Supporting information

Contents

Novel Isatin-triazole Based Thiosemicarbazones as Potential Anticancer agents: Synthesis, DFT and Molecular Docking Studies

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1 Instrumentation and materials

The melting points of the products were determined in open-end capillary tubes, by using Gallenkamp apparatus. Infrared spectra were recorded on Bruker Tensor II spectrophotometer (Germany) for functional group analysis. ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance 300 MHz NMR spectrophotometer (Switzerland) using deuterated solvents and the chemical shifts were calibrated relative to the residual solvent signal. *In vacuo* removal of solvents was performed using the Buchi rotary evaporator.

All the chemicals were obtained from Sigma Aldrich (Germany) and used as received. The solvents were distilled as per requirement using standard protocols. To monitor the progress of reaction, Thin Layer Chromatography (TLC) was performed by using pre-coated silica plates (Kieselgel-60 F_{254}, Merck, Germany). For product purification, flash chromatography was performed on silica gel (70-230 mesh).

2 Synthesis of 1-(2-chloroethyl)isatin (I)

Compound **I** was synthesized by following a reported modified procedure.[1] Powdered potassium carbonate (0.27 g, 4.9 mmol) was added to a stirred solution of isatin (0.50 g, 3.4 mmol) in DMF (10mL), then after 30 minutes the mixture was added to 1,2-dichloroethane (0.84g, 8.5mmol) and stirring was further continued at 60 ^{o}C for 24 hours. Progress of reaction was monitored by using TLC (n-hexane: EtOAc, 7:3). Upon completion of the reaction, cold distilled water (25 mL) was added to the reaction

mixture and crude product was extracted by using EtOAc (3x30 mL). The combined organic layers were concentrated by using rotavapor to obtain the crude product. Pure 1-(2-chloroethyl)indoline-2,3-dione was obtained through column chromatography (n-hexane: EtOAc, 85:15).

Appearance: Orange-Red Crystals; **Yield:** 73%; **Melting Point:** 110-112 ^{o}C (Lit. 111-112 ^{o}C)[2] ; R_f: 0.45 (n-hexane: EtOAc, 7:3); **FT-IR** $\boldsymbol{\nu}$ (cm^{-1}): 3064 (C_{sp2}-H stretch), 1731 (C=O stretch, ketone), 1609 (C=O stretch, lactam), 1484 (C=C stretch, aromatic ring), 1468 (C_{sp3}-H bend, methylene), 1341 (C-N stretch, lactam), 756 (C-H bend, 1,2-disubstitution), 656 (C-Cl stretch)

3 Synthesis of 1-(2-azidoethyl)isatin (1a)

To synthesize the 1-(2-azidoethyl)isatin, a reported procedure[3] was followed. Addition of compound **I** (0.20 g, 0.95 mmol) and sodium azide (0.07 g, 1.14 mmol) in DMF was carried out and stirred for 6 hours, maintaining the temperature at 60 ^{o}C. TLC was performed to monitor the reaction progress (n-hexane: EtOAc, 7:3). Upon completion of the reaction, the reaction mixture was poured onto crushed ice, bright orange precipitates were obtained which were washed with distilled water (20 mL) to yield the pure 1-(2-azidoethyl)isatin.

Appearance: Bright Orange Crystals; **Yield:** 92 % ; **Melting Point:** 79-81 ^{o}C; $\mathbf{R_f}$**:** 0.59 (n-hexane: EtOAc, 7:3); **FT-IR** $\boldsymbol{\nu}$ (cm^{-1}): 3068 (C_{sp2}-H stretch), 2940 (C_{sp3}-H stretch), 2107 (N=N=N stretch, azide) 1724 (C=O stretch, ketone), 1609 (C=O stretch, lactam), 1486 (C=C stretch, aromatic), 1468 (C_{sp3}-H bend, methylene), 1341 (C-N stretch, lactam), 753 (C-H bend, 1,2-disubstitution)

4 Synthesis of 4-(prop-2-ynyloxy)benzaldehyde (2a)

A modified reported procedure[4] was followed to synthesize the 4-(prop-2-ynyloxy)benzaldehyde **2a**. To a DMF (15mL) solution of 4-hydroxybenzaldehyde (0.50 g, 4.1 mmol) potassium carbonate (0.27 g, 4.8 mmol) was added and stirred for 30 minutes then dropwise addition of 3-bromoprop-1-yne (0.58 g, 4.9 mmol) was done and stirring was further continued at 60 ^{o}C. Thin layer chromatography was performed to monitor the reaction's progress (n-hexane: EtOAc, 7:3). Ice-cold distilled water (20 mL) was added upon completion and light brown precipitates obtained were filtered

off. Precipitates were washed with distilled water, dried and recrystallized from ethanol to yield pure 4-(prop-2-ynyloxy)benzaldehyde.

Appearance: Light Brown Crystals; **Yield:** 86 %; **Melting Point:** 83-84 ^{o}C (Lit. 82-84 ^{o}C)[5]; R_f: 0.63 (n-hexane: EtOAc, 7:3); **FT-IR** $\boldsymbol{\nu}$ (cm^{-1}): 3205 (C_{sp}-H stretch), 2970 (C_{sp2}-H stretch), 2833 (C_{sp3}-H stretch), (2808, 2750) (C_{sp2}-H stretch, aldehyde), 2110 (C≡C stretch), 1679 (C=O stretch, aldehyde), 1574 (C=C stretch, aromatic ring), 1229 (C_{sp2}-O stretch, ether), 1169 (C_{sp3}-O stretch, ether), 833 (C-H bend, 1,4-disubtitution)

5 Synthesis of 1-(prop-2-ynyl)indoline-2,3-dione (1b)

A reported procedure[4] was followed for the synthesis of **1b**, in which isatin (0.50 g, 3.4 mmol) and potassium carbonate (0.52 g, 4.08 mmol) were dissolved in DMF (15 mL) and stirred for 30 minutes , followed by the dropwise addition of 3-bromoprop-1-yne (0.42 g, 3.4 mmol). Stirring was further continued for about 3 hours at room temperature and reaction's progress was monitored through TLC (n-hexane: EtOAc, 8:2). After the reaction completion, cold water was added to the reaction mixture, and orange precipitates obtained were filtered off, washed with distilled water. Pure crystals of **1b** were obtained through recrystallization from ethanol.

Appearance: Orange Crystals; **Yield**: 92 %; **Melting Point**: 150-153 ^{o}C; $\mathbf{R_f}$: 0.69 (n-hexane: EtOAc, 8:2); **FT-IR** $\boldsymbol{\nu}$ (cm^{-1}): 3262 (C_{sp}-H, alkyne), 3064 (C_{sp2}-H stretch), 2963 (C_{sp3}-H stretch), 2130 (C≡C stretch), 1734 (C=O stretch, ketone), 1614 (C=O stretch, lactam), 1598 (C=C stretch, aromatic ring), 1463 (C_{sp3}-H bend, methylene), 1342 (C-N stretch, lactam), 761 (C-H bend, 1,2-disubstitution); **^{1}H NMR** (300 MHz, $CDCl_3$) δ (ppm): 7.69-7.64 (m, 2H, Ar-H), 7.22-7.13 (m, 2H, Ar-H), 4.55 (d, ^{3}J = 2.4 Hz, 2H, -CH_2), 2.38 (t, ^{3}J = 2.4 Hz, 1H, -C≡CH); **^{13}C NMR** (75 MHz, $CDCl_3$) δ (ppm): 182.5, 157.1, 149.6, 138.4, 125.5, 124.2, 117.6, 111.1, 75.6, 73.3, 29.4

6 Synthesis of 4-(2-chloroethoxy)benzaldehyde (II)

A reported modified procedure[1] was utilized for the synthesis of **II**. Powdered potassium carbonate (0.27 g, 4.9 mmol) was added to a stirred solution of 4-hydroxybenzaldehyde (0.50 g, 4.1 mmol) in CH_3CN:DMF (9:1), then after 30 minutes the mixture was added to 1,2-dichloroethane (1.01 g, 10.2 mmol) and stirring was further continued at 85 ^{o}C for 24 hours. Progress of reaction was monitored by using

TLC (n-hexane: EtOAc, 7:3). Upon completion of the reaction, cold distilled water (25 mL) was added to the reaction mixture and crude product was extracted using EtOAc (3x30 mL), washed with distilled water (20 mL) and concentrated under vacuum. Pure 4-(2-chloroethoxy)benzaldehyde was obtained through column chromatography (n-hexane: EtOAc, 85:15) which solidified in refrigerator.

Appearance: Transparent Liquid; **Yield:** 63 %; **Melting Point:** 25 °C; $\mathbf{R}_f$: 0.64 (n-hexane: EtOAc, 7:3)

7 Synthesis of 4-(2-azidoethoxy)benzaldehyde (2b)

To synthesize the 4-(2-azidoethoxy)benzaldehyde, addition of **2b** (0.50 g, 2.7 mmol) and sodium azide (0.26 g, 4.0 mmol) were done in DMF (15mL). This mixture was stirred for 6 hours at 60 °C and the progress of reaction was monitored by TLC (n-hexane: EtOAc, 7:3). Upon the completion of the reaction, ice-cold distilled water (15 mL) was added to the reaction mixture, extraction with EtOAc (3x20 mL) was done and washed with distilled water. Pure 4-(2-azidoethoxy)benzaldehyde was obtained by concentrating the organic layers under vacuum by rotary evaporation.

Appearance: Transparent Liquid; **Yield**: 78 %; $\mathbf{R}_f$: 0.63 (n-hexane: EtOAc, 7:3)

8 General procedure for the synthesis of N^4-substituted thiosemicarbazide ($\mathbf{3c_1}$-$\mathbf{3c_4}$)

For the synthesis of N^4-substituted thiosemicarbazides $\mathbf{3c_1}$-$\mathbf{3c_4}$, respective isothiocyanate (1g, 7.4mmol) was dissolved in ethanol (10ml) in a flask at constant stirring in an ice bath, then added the hydrazine hydrate (0.9g, 18mmol) in stirring isothiocyanate solution and vigorous stirring was continued for 2 hrs in ice bath. After the completion of reaction as indicated by TLC, precipitates were filtered off and ethanol was removed from the filtrate through rotary evaporator.

8.1 4-Phenylthiosemicarbazide ($\mathbf{3c_1}$)

Appearance: White Crystals; **Yield:** 92 %; **Melting Point:** 138-140 °C; $\mathbf{R}_f$**:** 0.42 (n-hexane: EtOAc, 4:6); **^{1}H NMR** (300 MHz, $CDCl_3$) δ (ppm): 9.78 (s, 1H, -NH), 9.26 (s, 1H, -NH), 7.76 (d, 2H, ^3J = 6Hz, Ar-H), 7.34 (t, 2H, ^3J = 9Hz, Ar-H), 7.16 (t, 2H,

^{3}J = 6.9Hz, Ar-H), 3.98(s, 2H, -NH_2); **^{13}C NMR** (75 MHz, $CDCl_3$) δ (ppm): 176.7, 150.6, 139.1, 128.1, 124.8, 123.8, 123.7

8.2 4-(*p*-Tolyl)thiosemicarbazide (3c $_2$)

Appearance: Off-white Crystals; **Yield:** 82 %; **Melting Point:** 130-133 ^{o}C; **R_f:** 0.40 (n-hexane: EtOAc, 4:6); **^{1}H NMR** (300 MHz, $CDCl_3$) δ (ppm): 9.18 (s, 1H, -NH), 8.43 (s, 1H, -NH), 7.41 (d, 2H, ^{3}J = 6Hz, Ar-H), 7.19 (d, 2H, ^{3}J = 6Hz, Ar-H), 3.98 (s, 2H, -NH_2), 2.35 (s, 3H, -CH_3); **^{13}C NMR** (75 MHz, $CDCl_3$) δ (ppm): 180.7, 135.8, 135.1, 129.4, 125.6, 124.6, 21.1

8.3 4-(4-Nitrophenyl)thiosemicarbazide (3c $_3$)

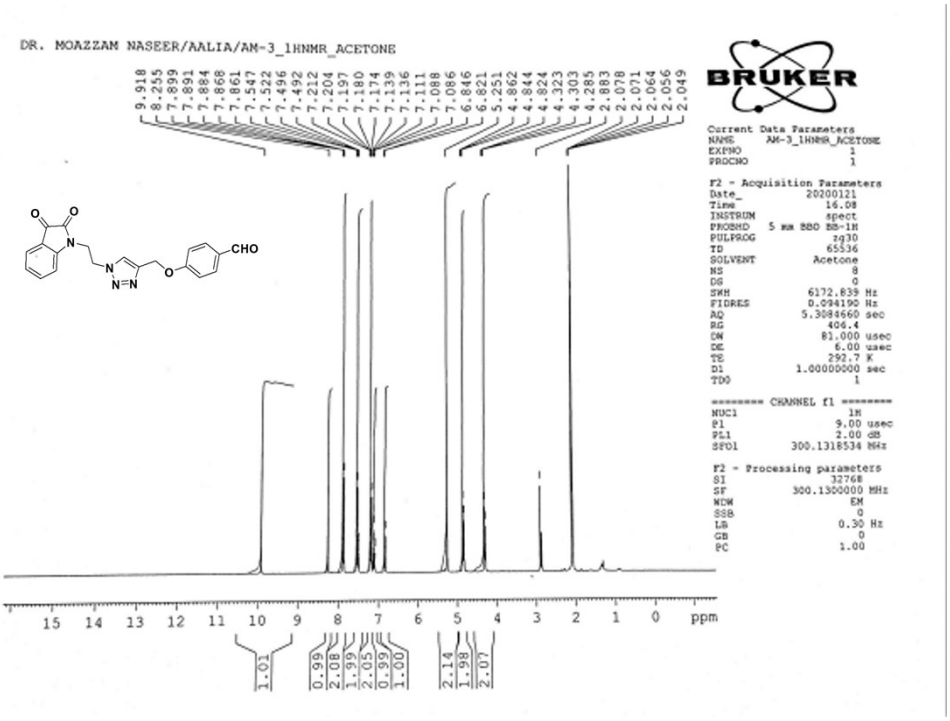
Appearance: Orange Crystals; **Yield**: 86%; **Melting Point:** 185 ^{o}C; **R_f:** 0.39 (n-hexane: EtOAc, 4:6); **^{1}H NMR** (300 MHz, $CDCl_3$) δ (ppm): 9.18 (s, 1H, -NH), 8.43 (s, 1H, -NH), 7.41 (d, 2H, ^{3}J = 6Hz, Ar-H), 7.19 (d, 2H, ^{3}J = 6Hz, Ar-H), 3.98 (s, 2H, -NH_2); **^{13}C NMR** (75 MHz, $CDCl_3$) δ (ppm): 180.7, 135.8, 135.1, 129.4, 125.6, 124.6

8.4 4-(4-Cyanophenyl)thiosemicarbazide (3c $_4$)

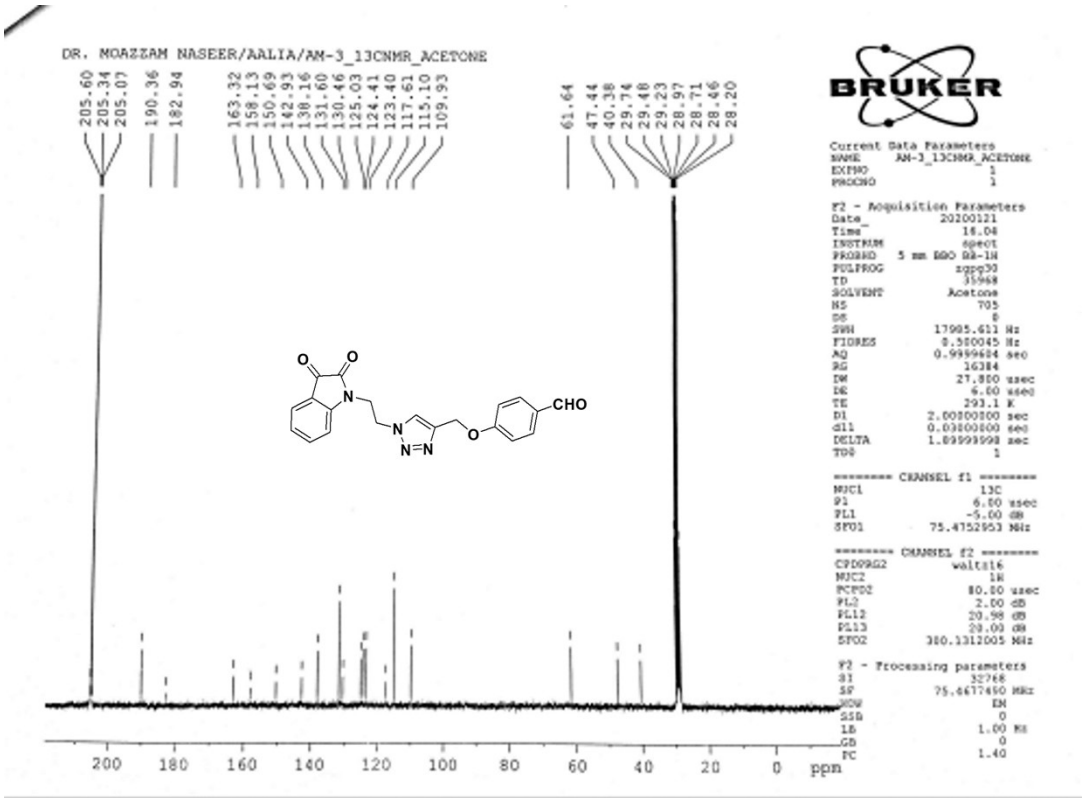
Appearance: White Crystals; **Yield:** 91%; **Melting Point:** 194 ^{o}C; **R_f**: 0.37 (n-hexane: EtOAc, 4:6); **Appearance:** Orange Crystals; **Yield**: 86%; **Melting Point:** 185 ^{o}C; **R_f:** 0.39 (n-hexane: EtOAc, 4:6); **^{1}H NMR** (300 MHz, $CDCl_3$) δ (ppm): 9.18 (s, 1H, -NH), 8.43 (s, 1H, -NH), 7.41 (d, 2H, ^{3}J = 6Hz, Ar-H), 7.19 (d, 2H, ^{3}J = 6Hz, Ar-H), 3.98 (s, 2H, -NH_2); **^{13}C NMR** (75 MHz, $CDCl_3$) δ (ppm): 180.72, 135.85, 135.13, 129.42, 125.62, 124.69, 118.9

9 NMR spectroscopy

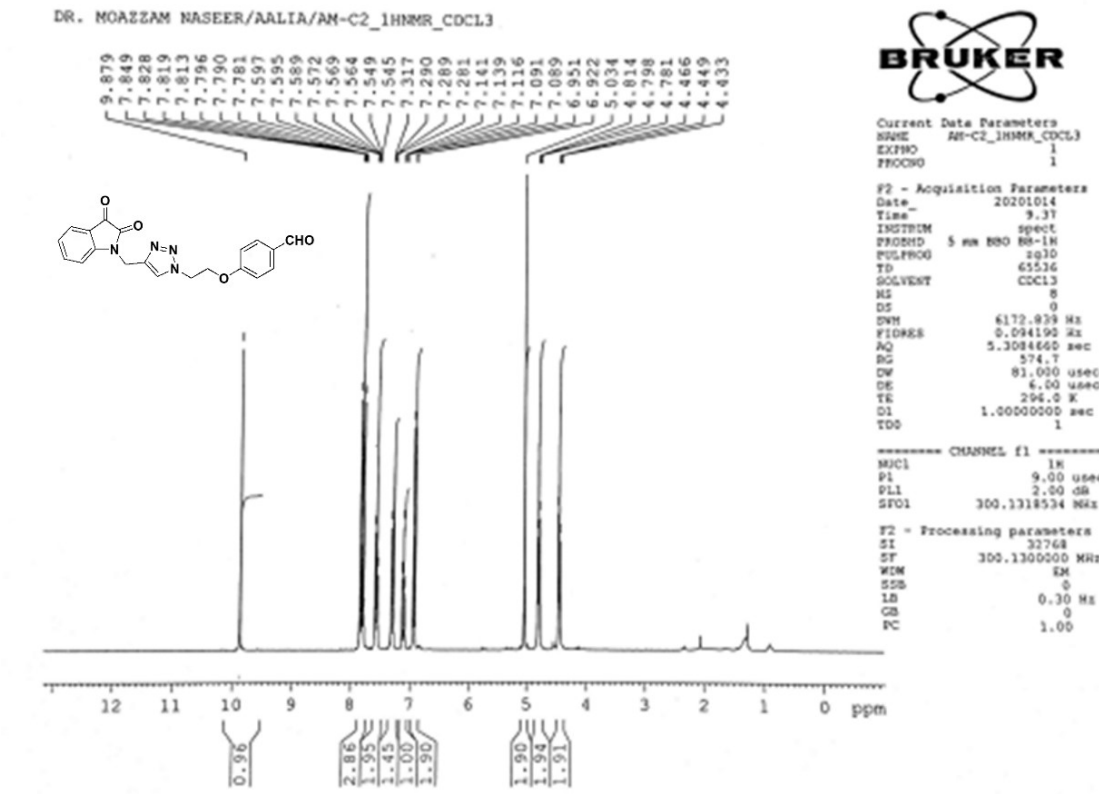
^{1}H NMR spectrum of compound 3a

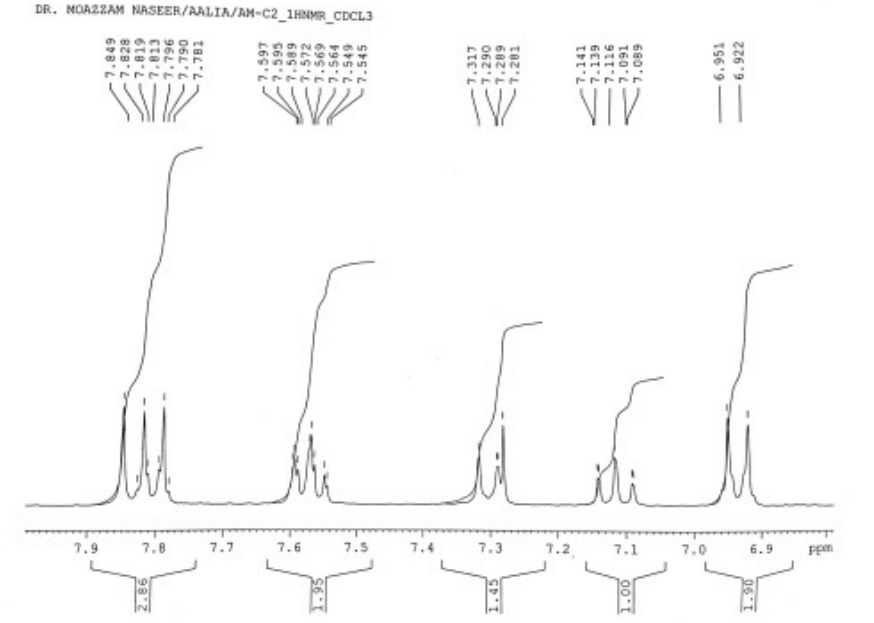


^{13}C NMR spectrum of compound 3a

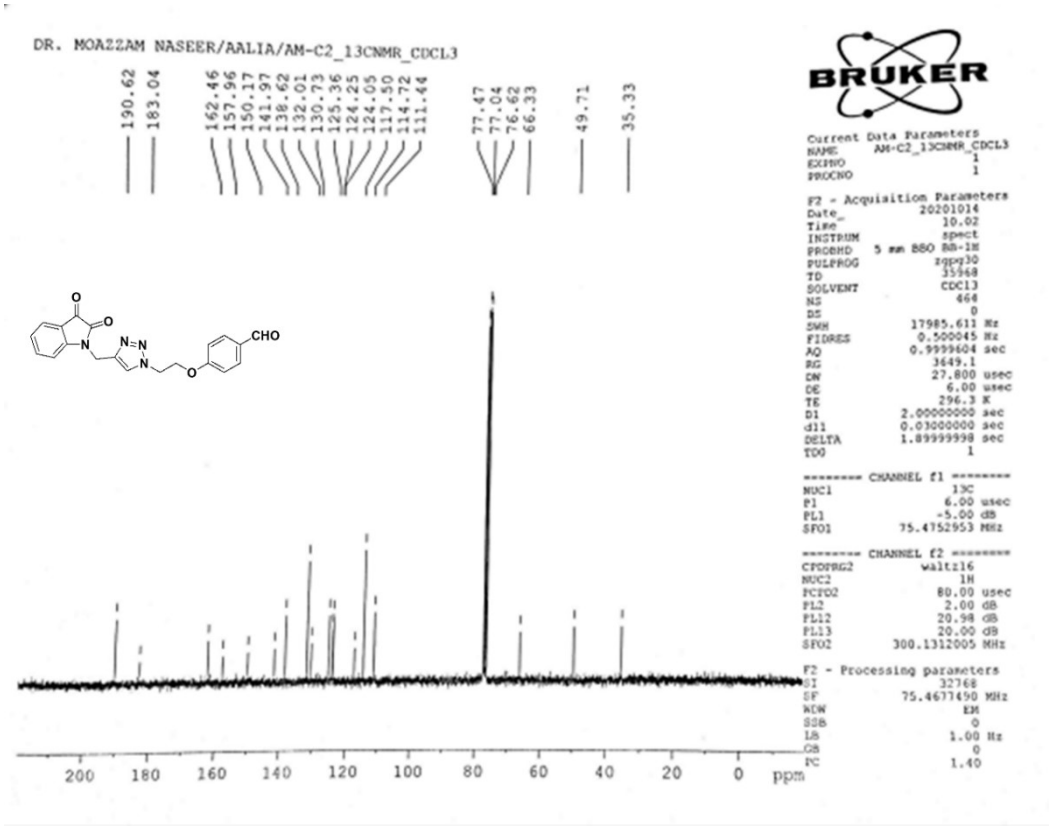


^{1}H NMR spectrum of compound 3b

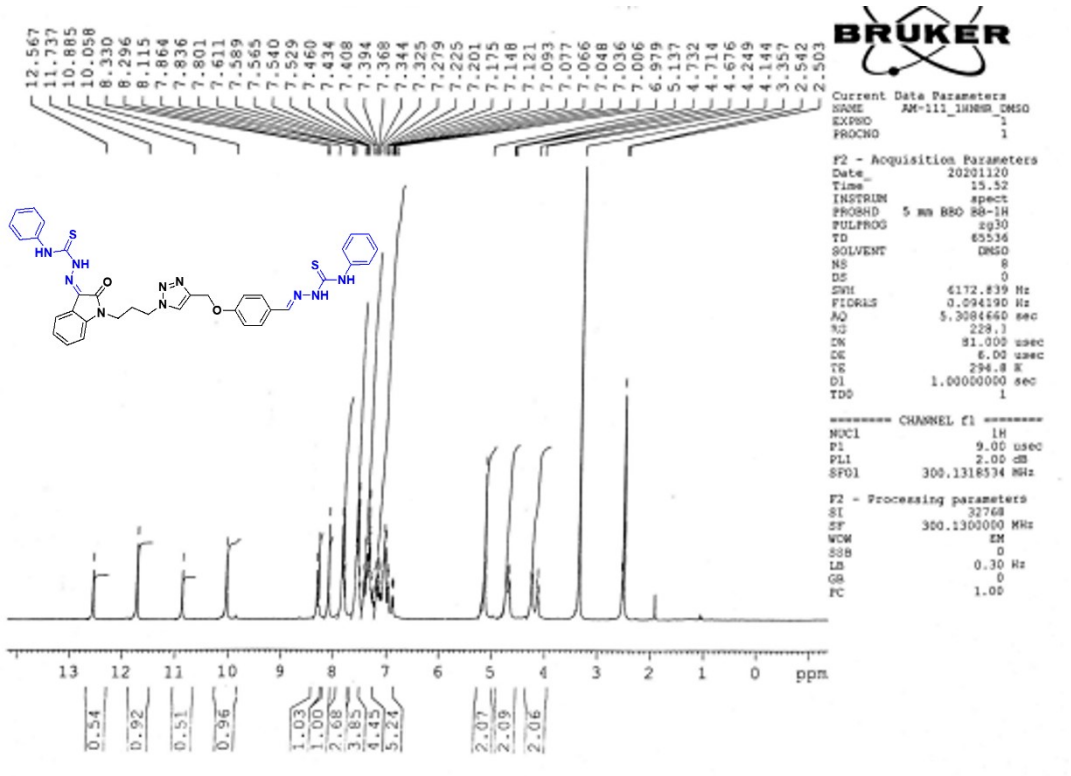




^{13}C NMR spectrum of compound 3b



^{1}H NMR spectrum of compound 5a



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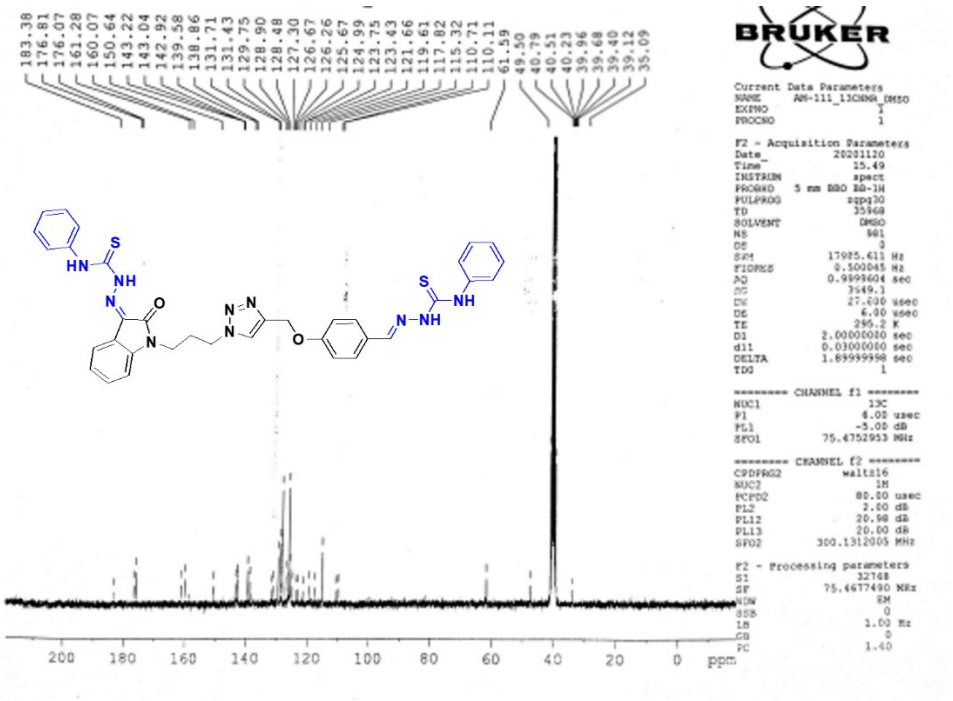

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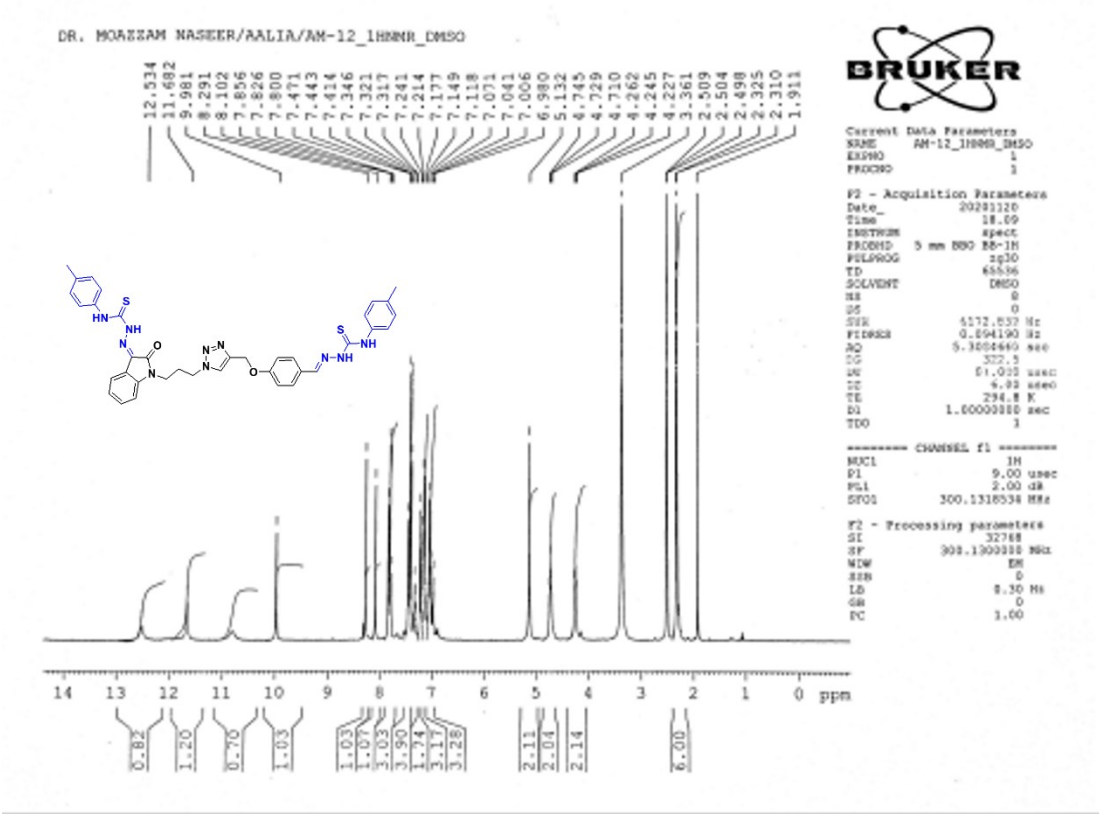
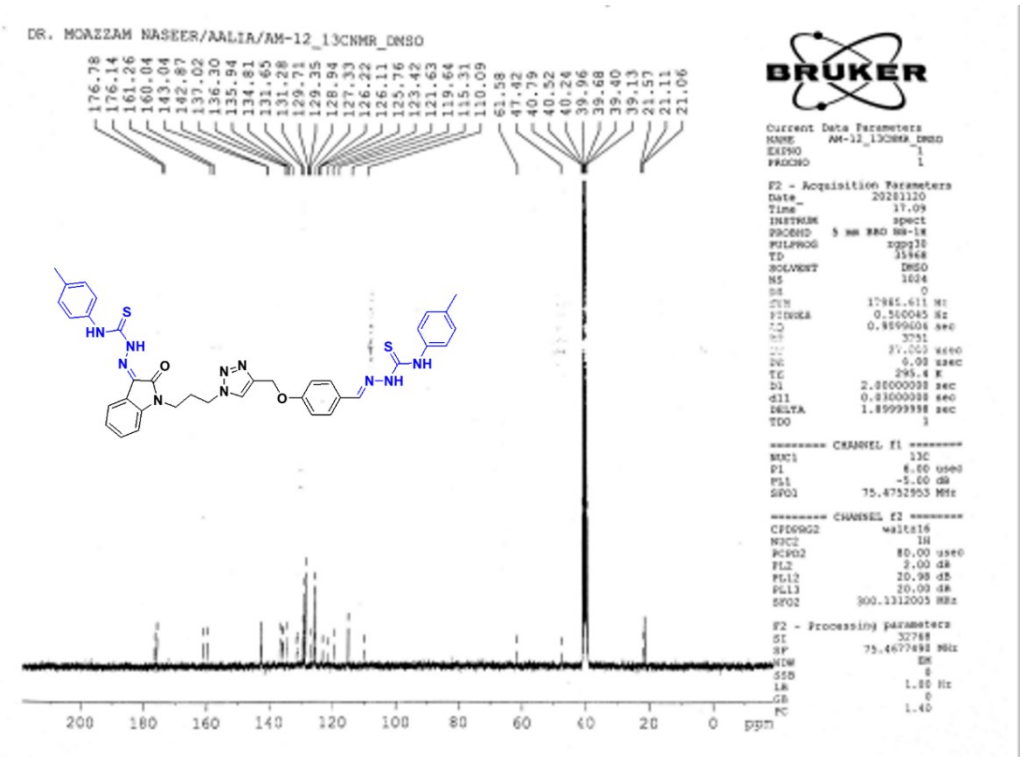
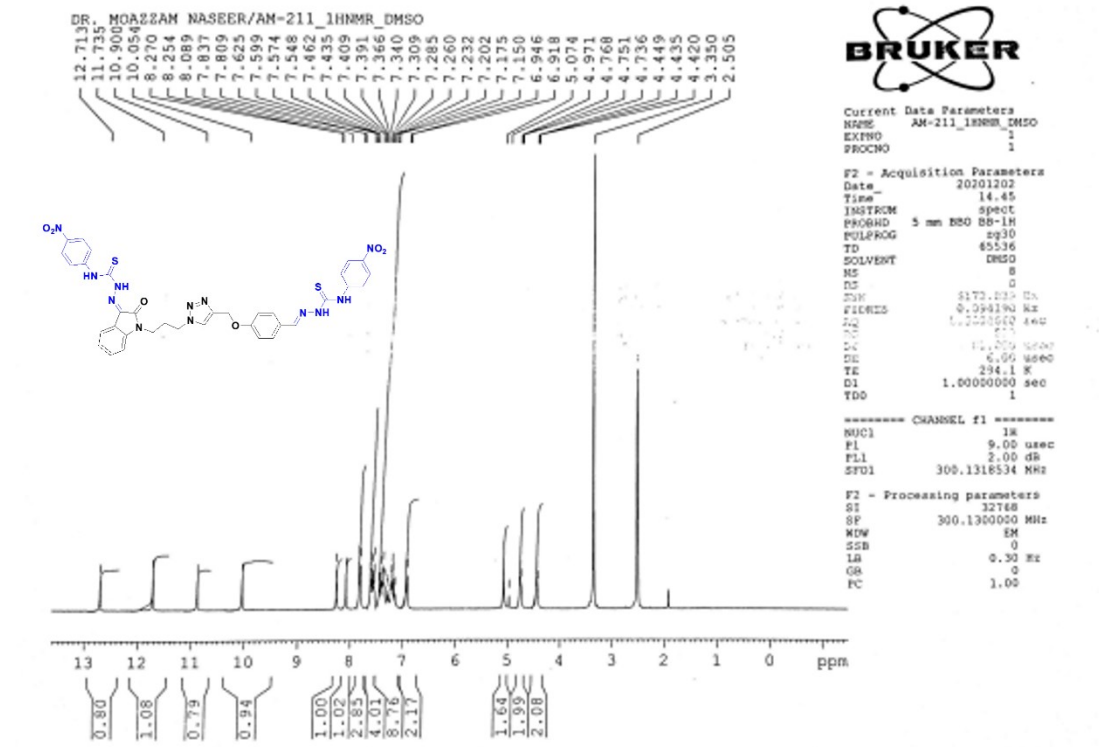


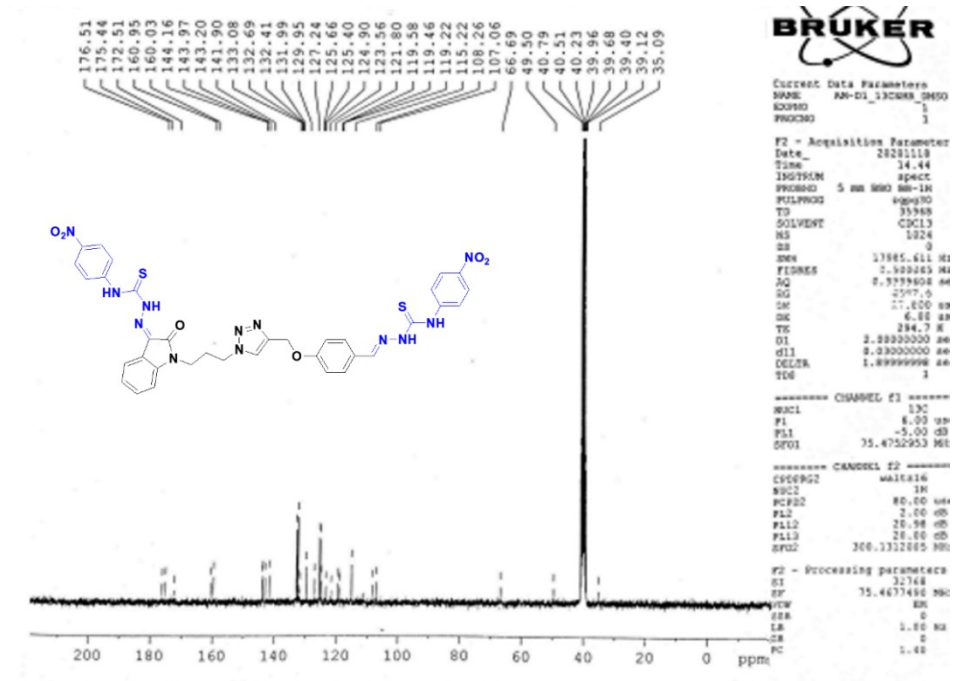
^{13}C NMR spectrum of compound 5b



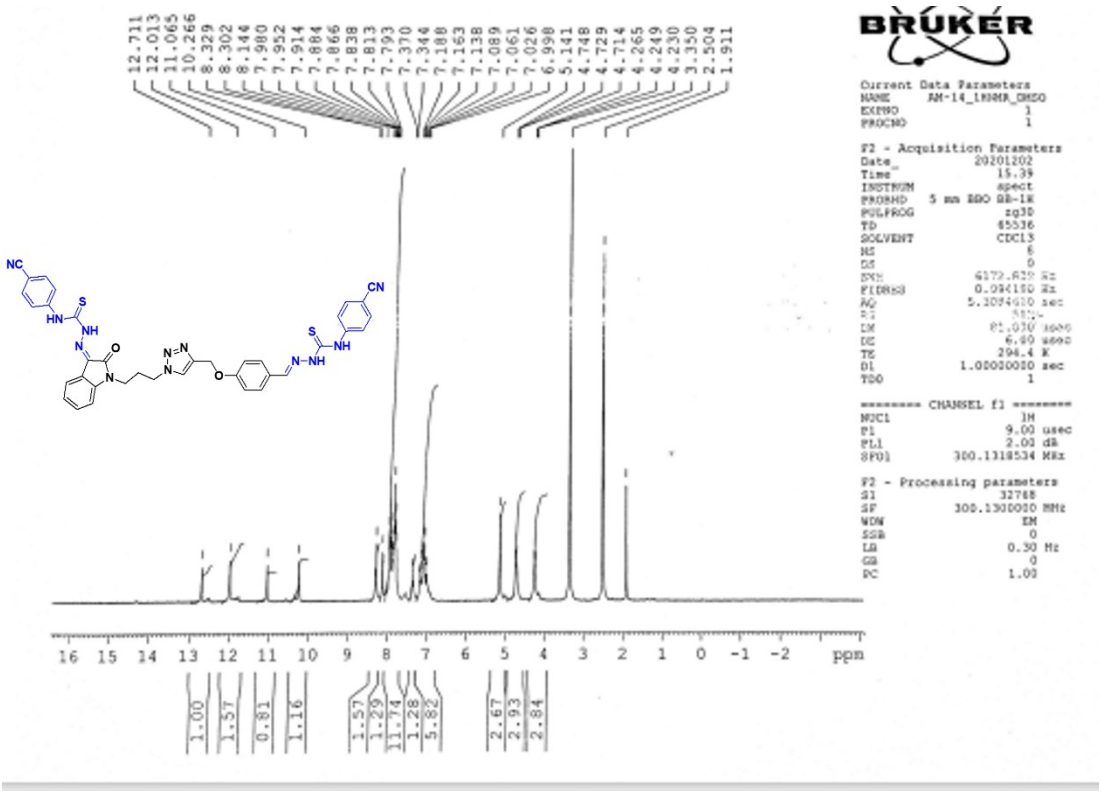
^{1}H NMR spectrum of compound 5c



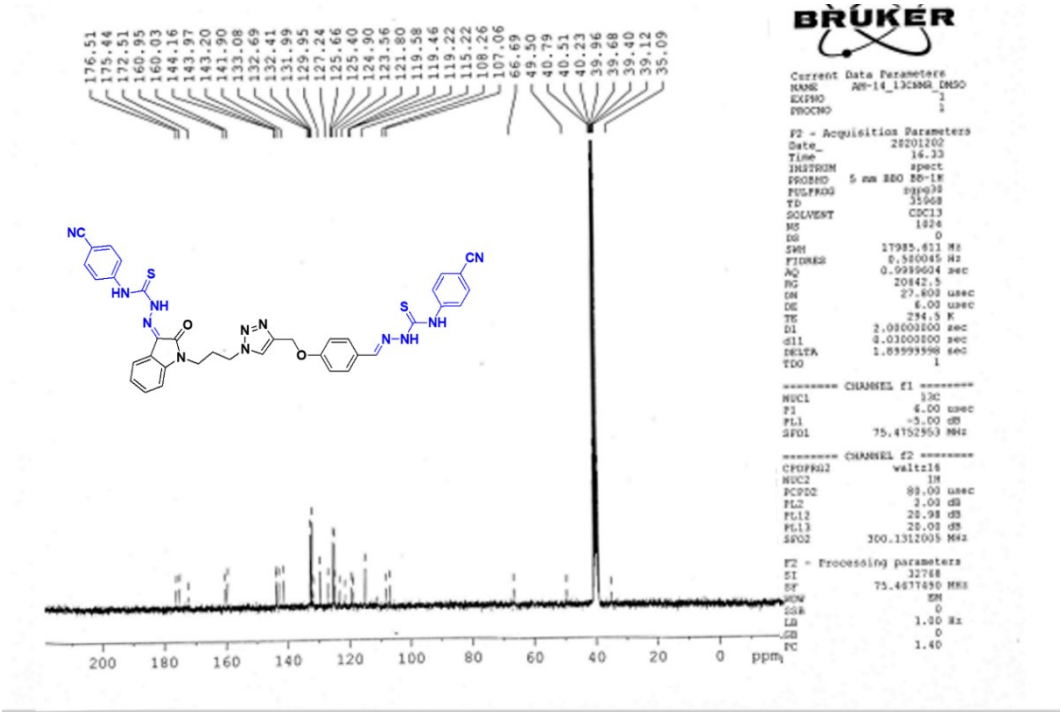
^{13}C NMR spectrum of compound 5c



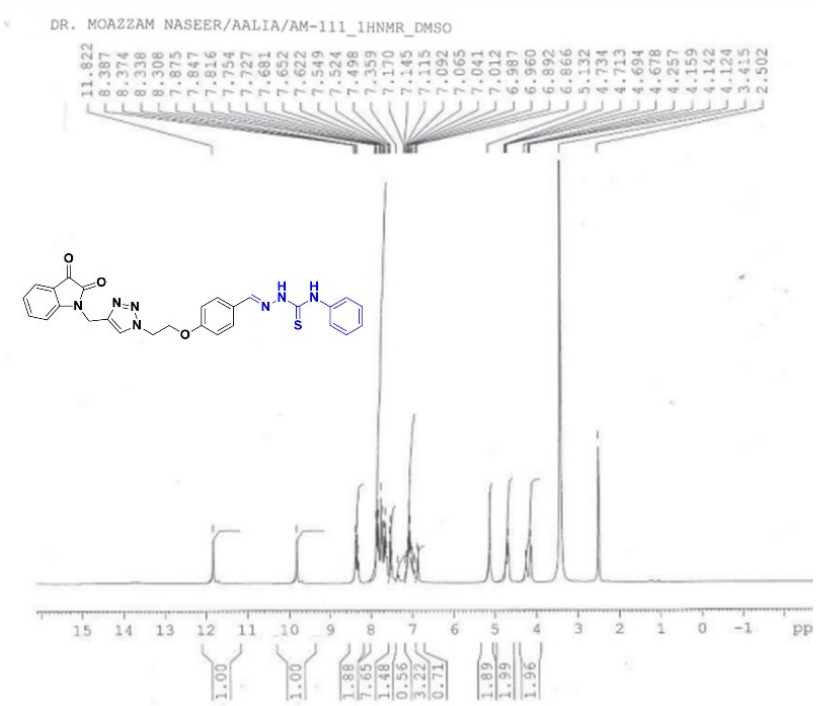
^{1}H NMR spectrum of compound 5d



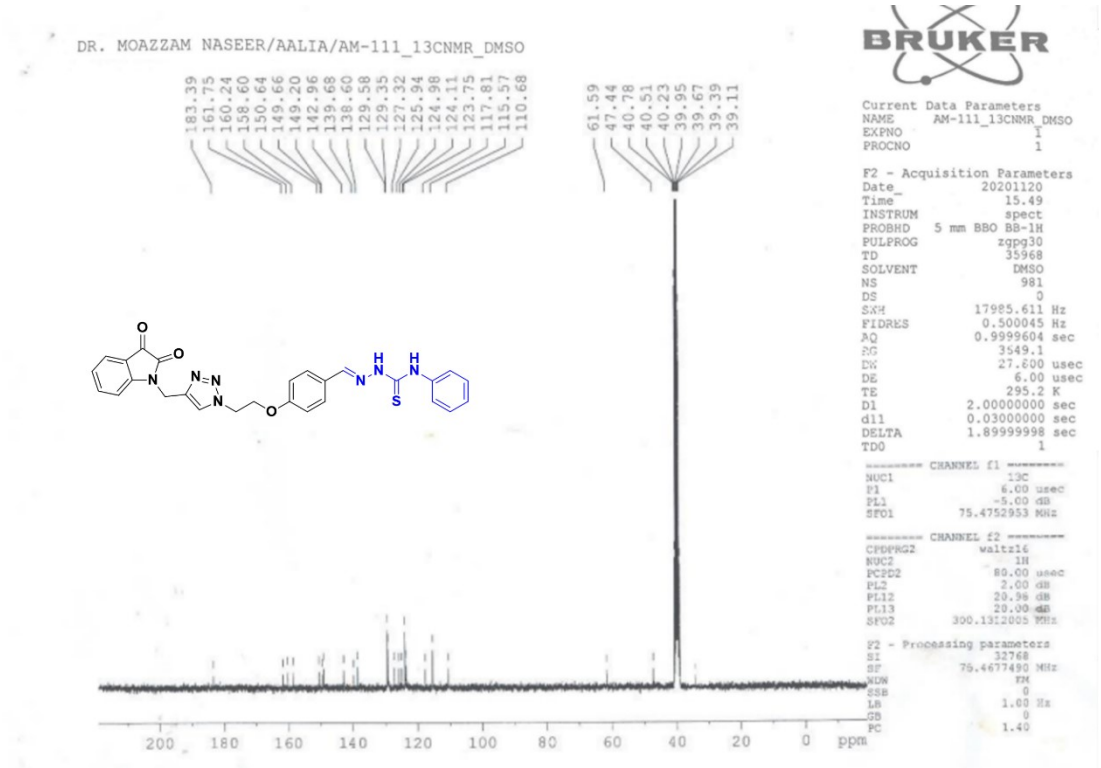
^{13}C NMR spectrum of compound 5d



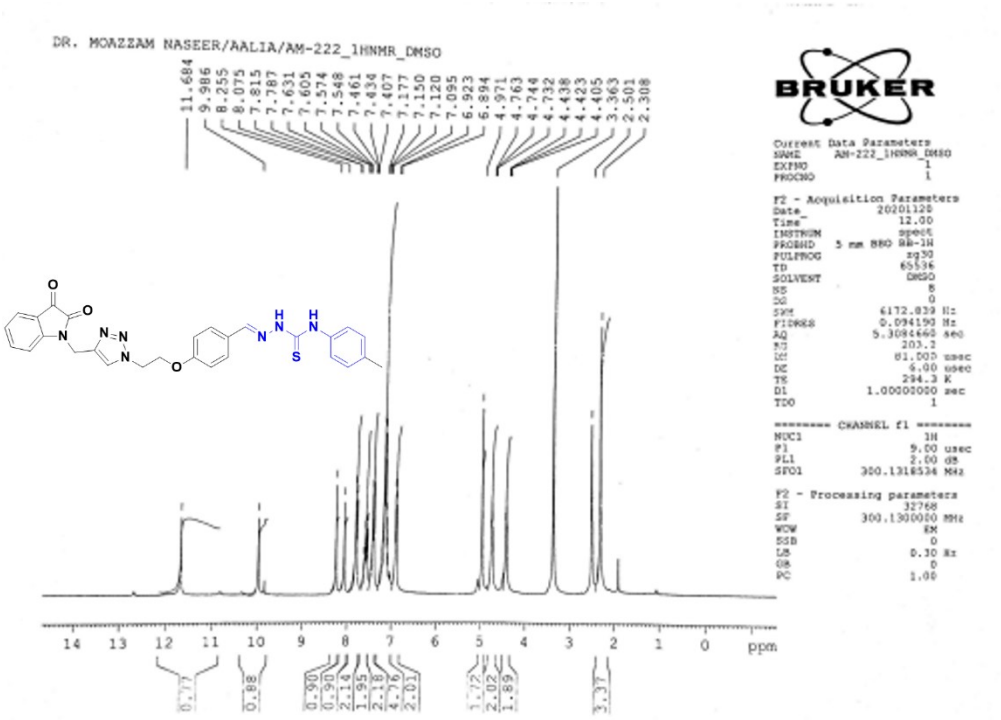
^{1}H NMR spectrum of compound 4e

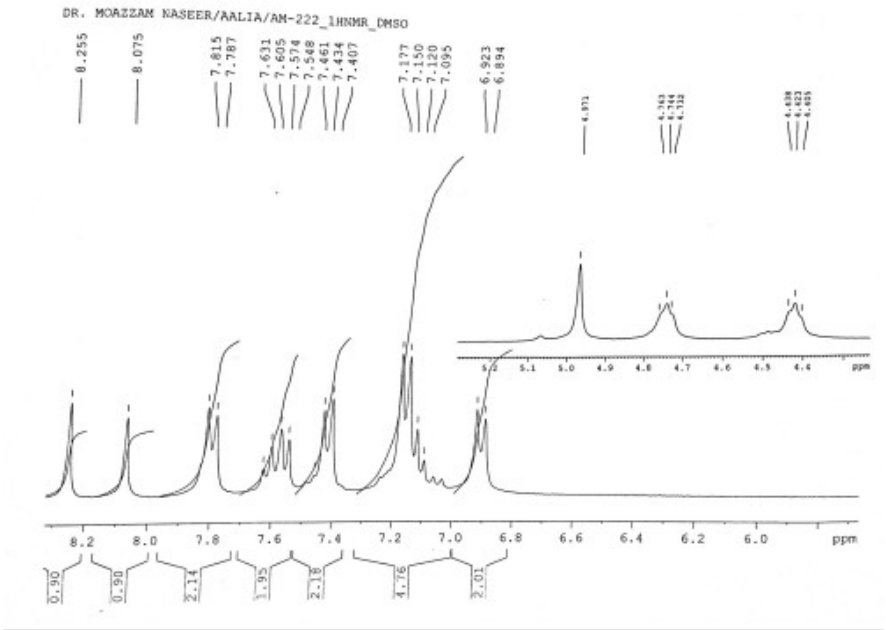


^{13}C NMR spectrum of compound 4e

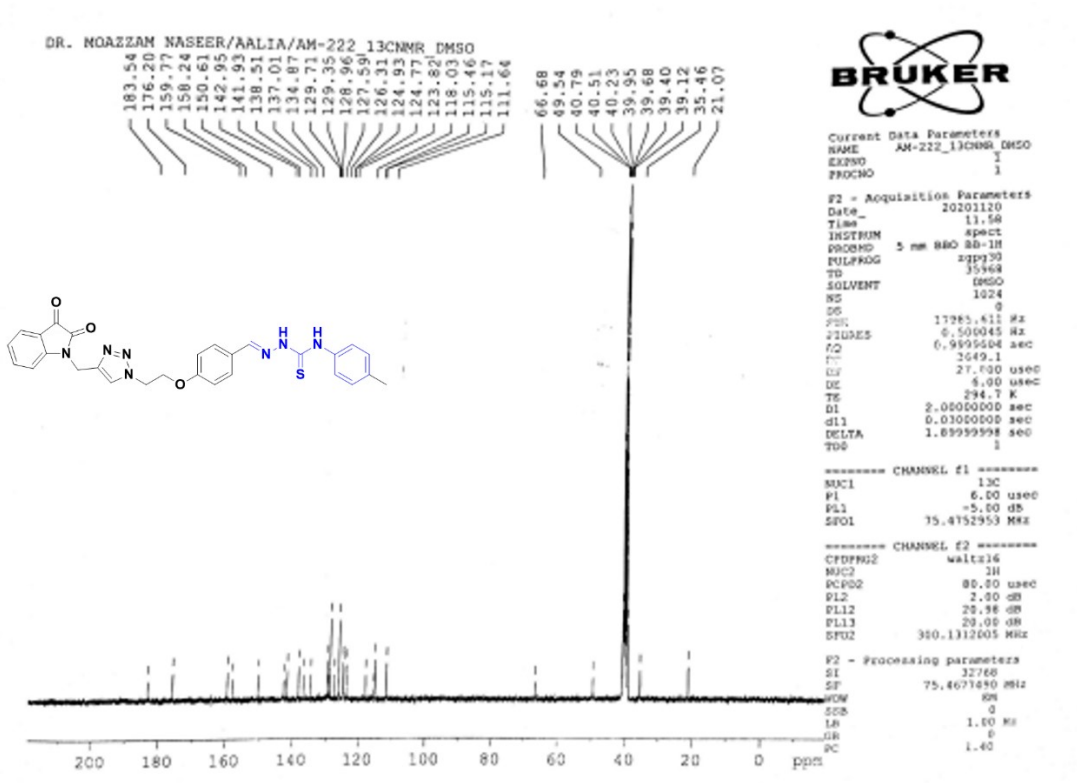


^{1}H NMR spectrum of compound 4f

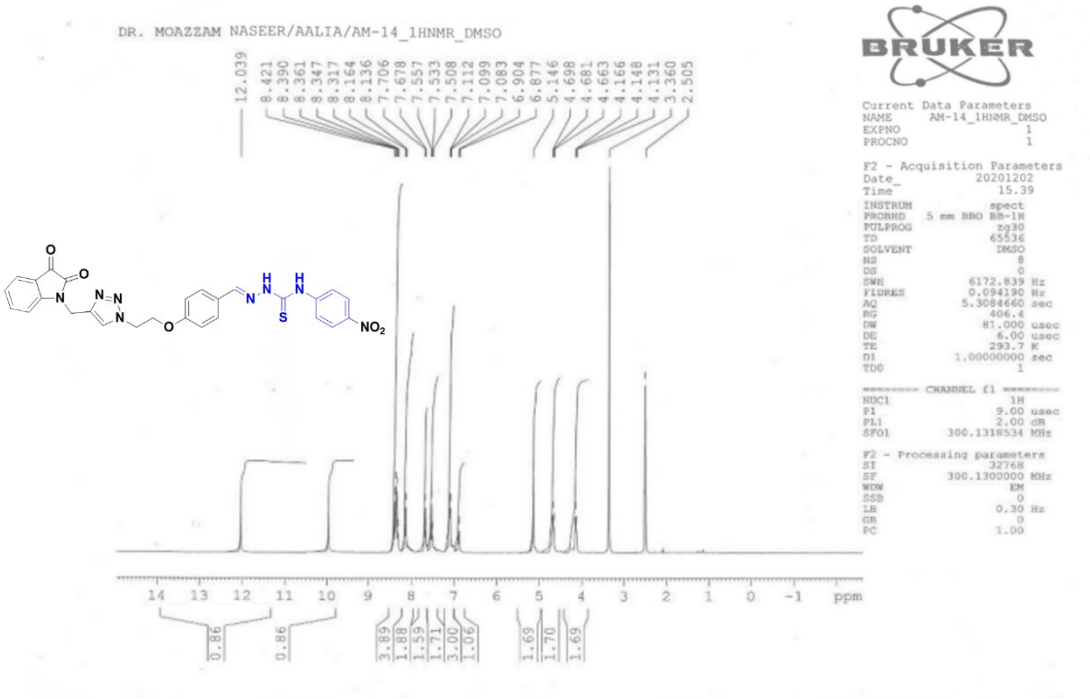




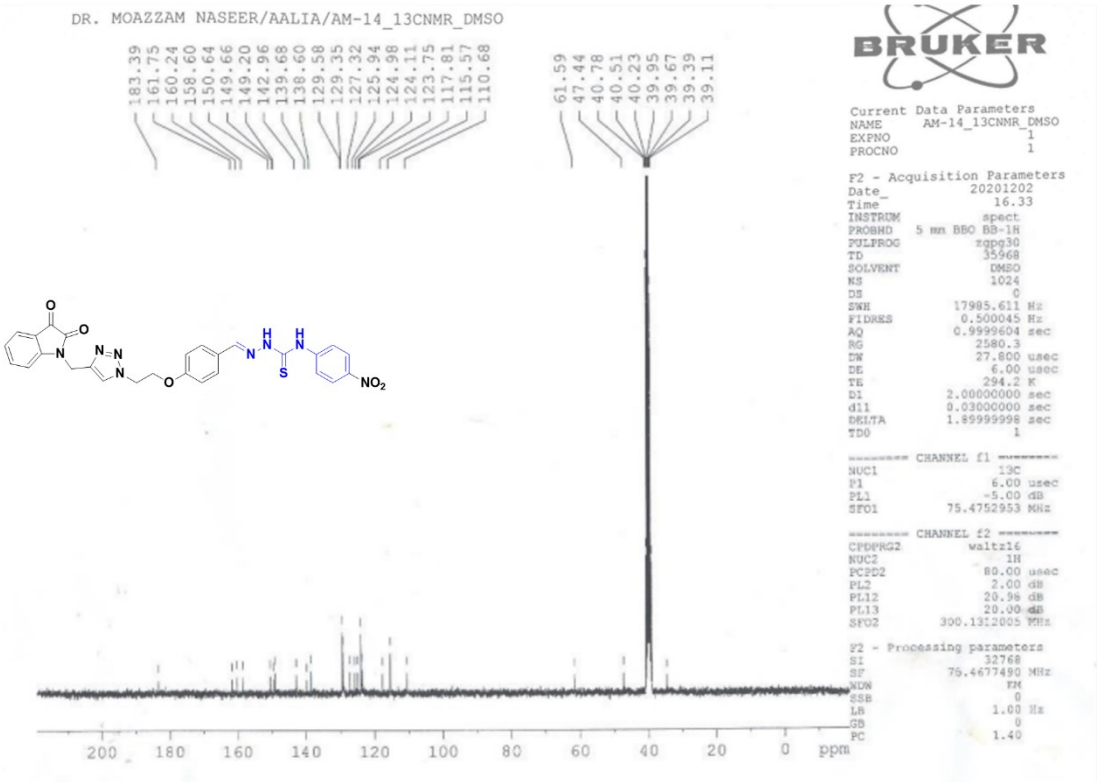
^{13}C NMR spectrum of compound 4f



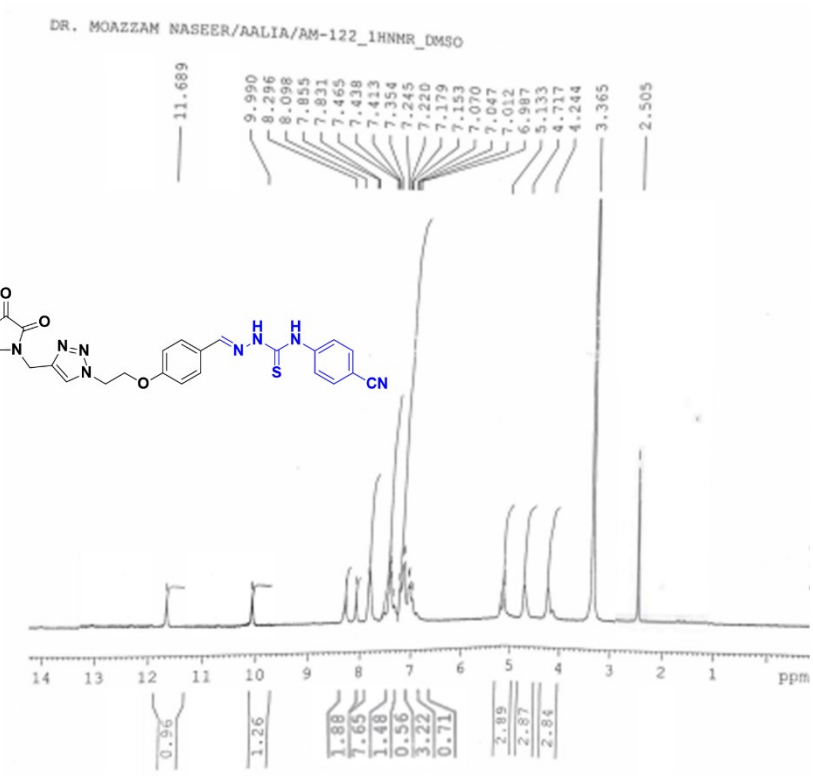
^{1}H NMR spectrum of compound 4g



^{13}C NMR spectrum of compound 4g



^{1}H NMR spectrum of compound 4h



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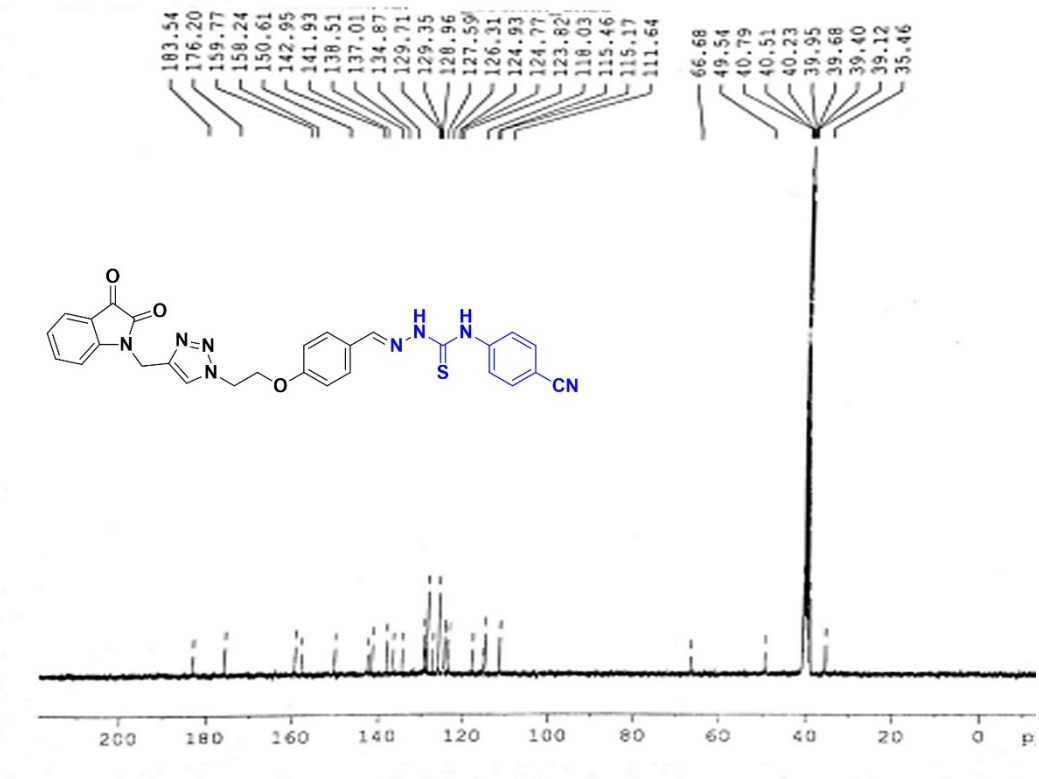
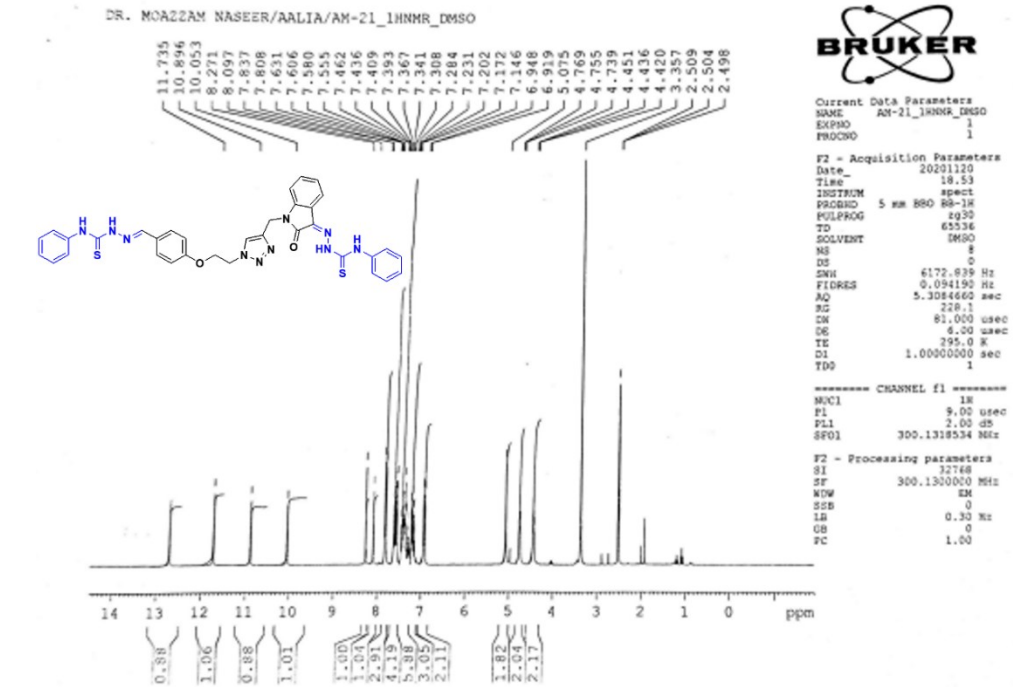
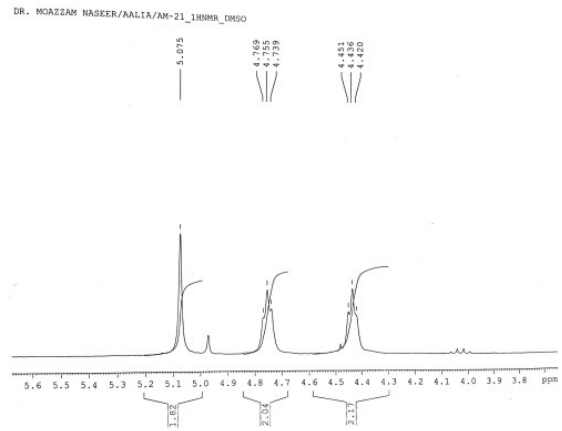
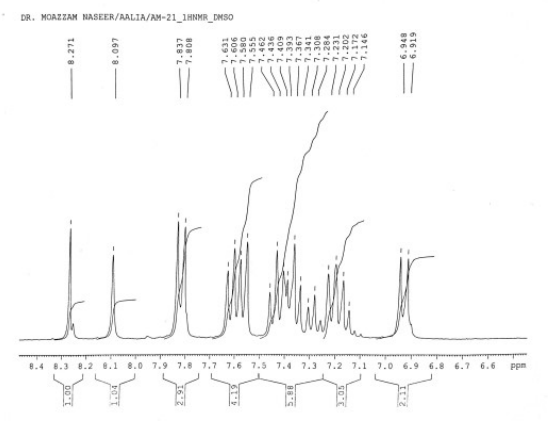


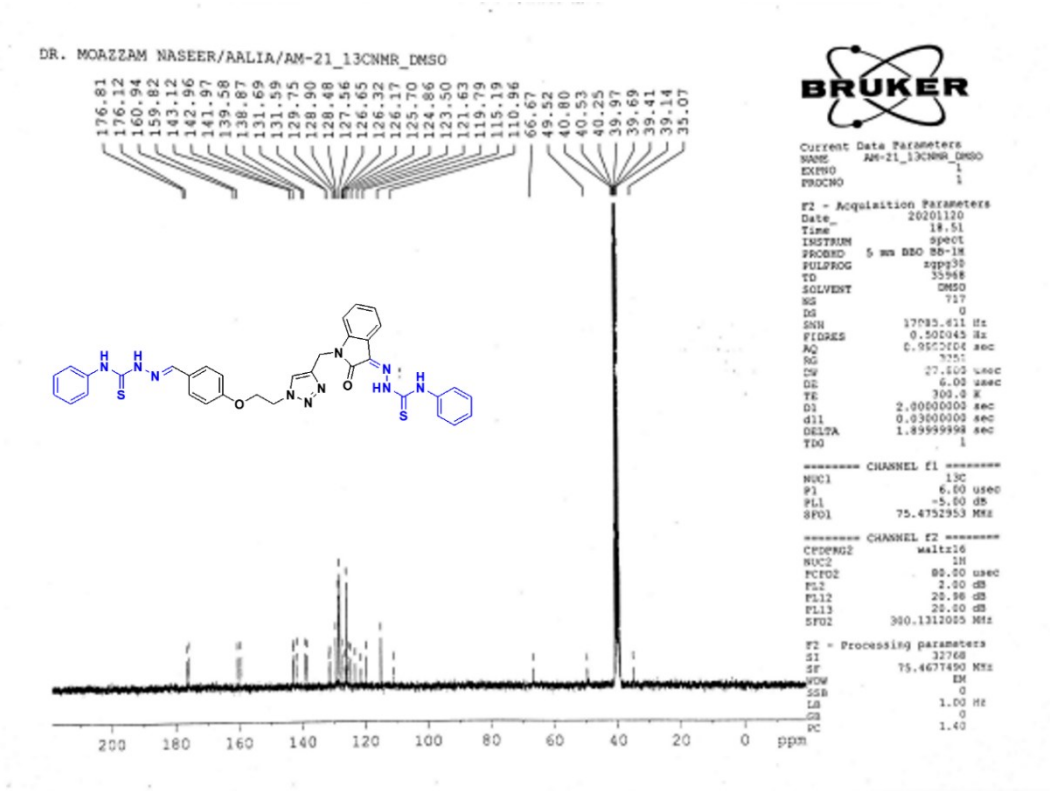
^{1}H NMR spectrum of compound 5e



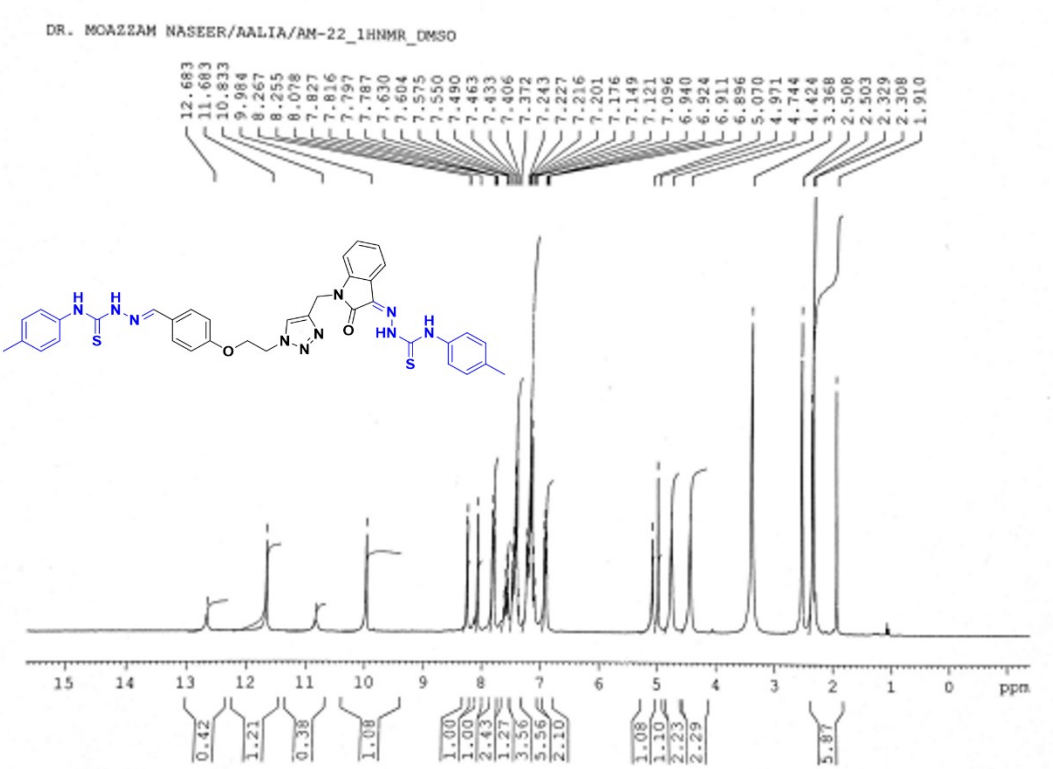




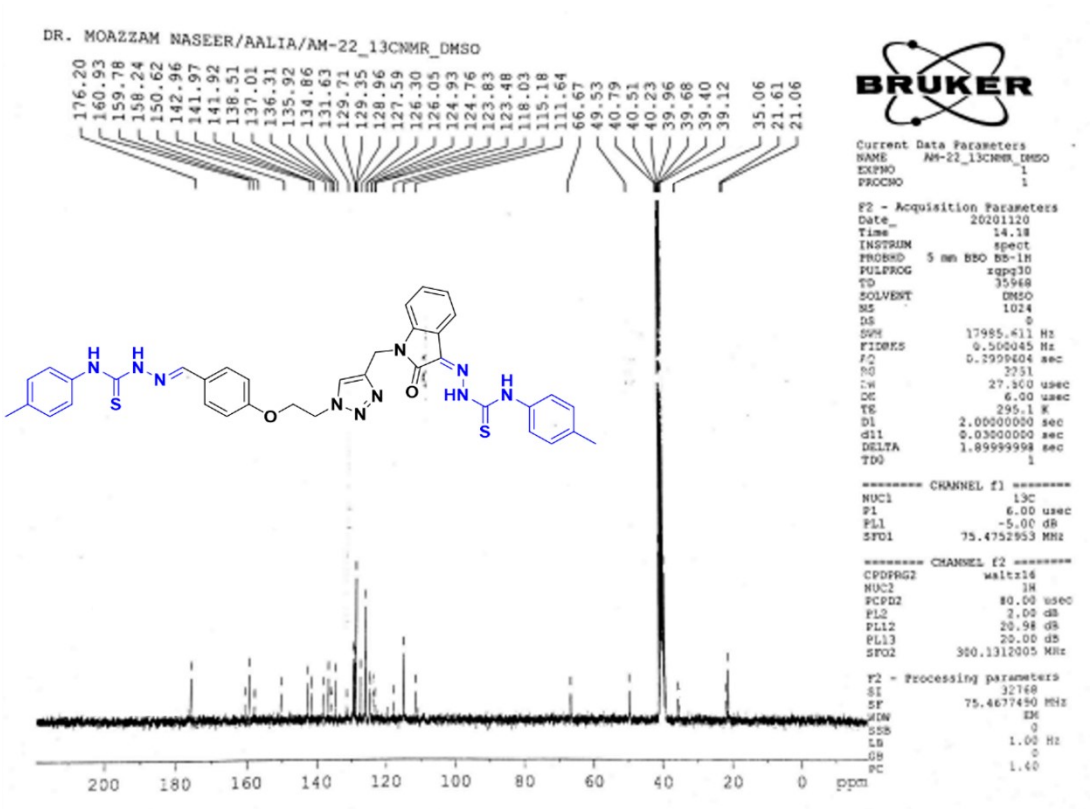
^{13}C NMR spectrum of compound 5e



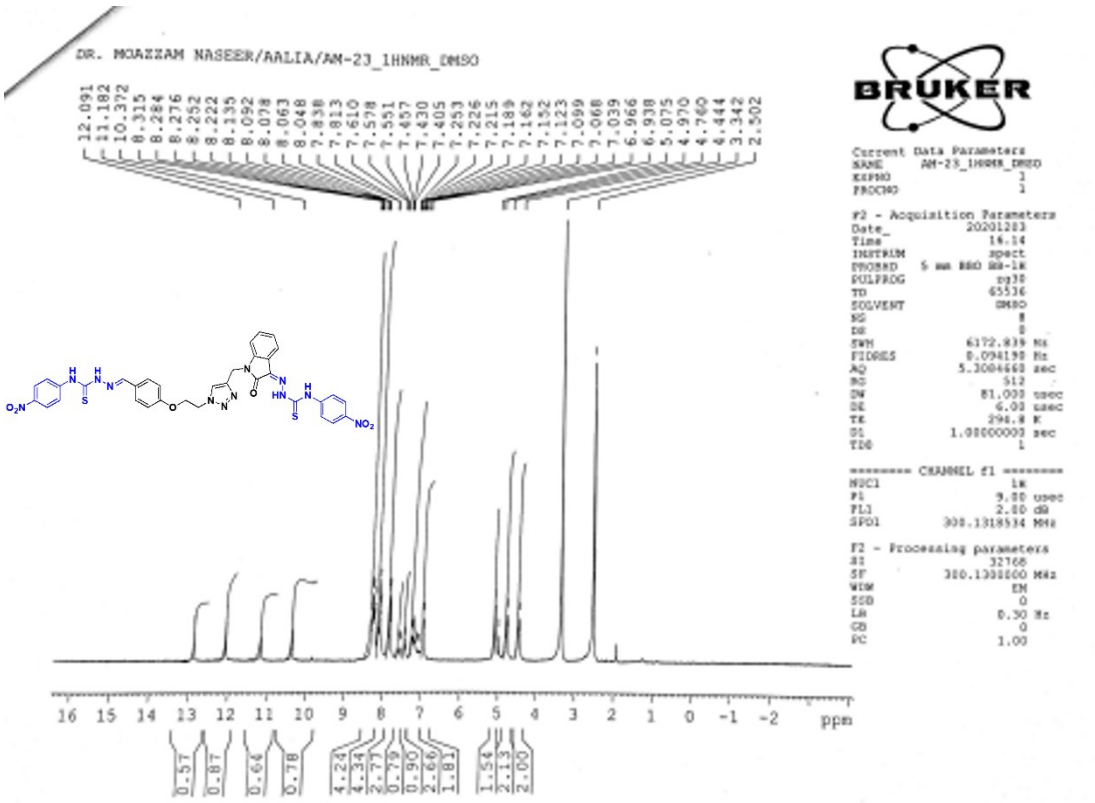
^{1}H NMR spectrum of compound 5f



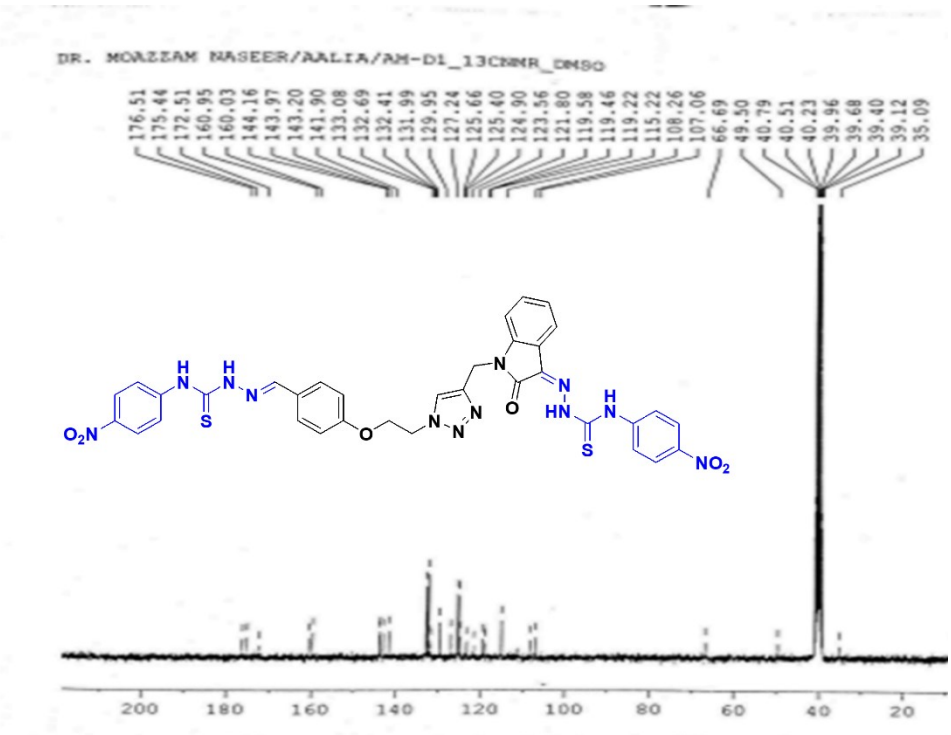
^{13}C NMR spectrum of compound 5f



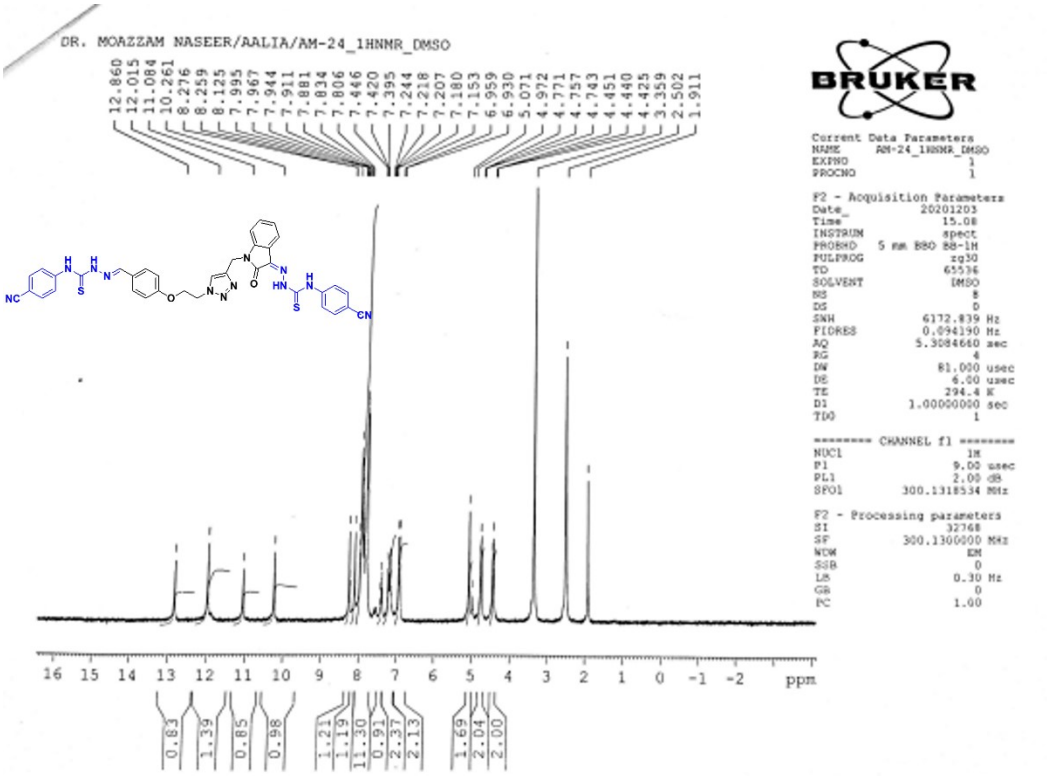
^{1}H NMR spectrum of compound 5g



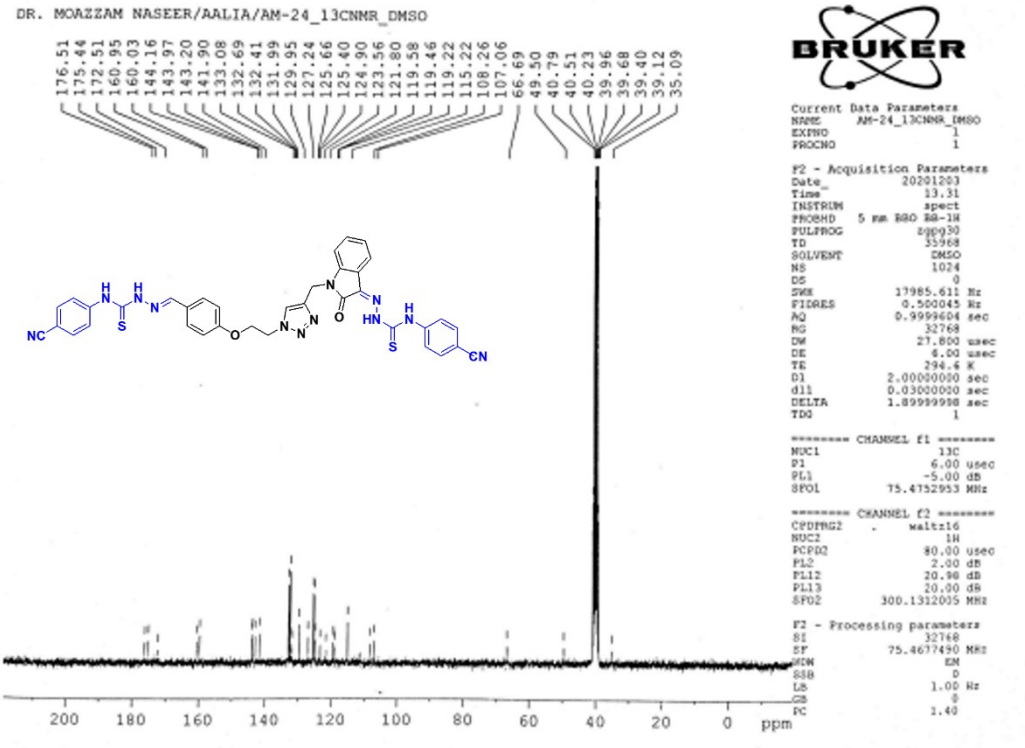
^{13}C NMR spectrum of compound 5g



^{1}H NMR spectrum of compound 5h

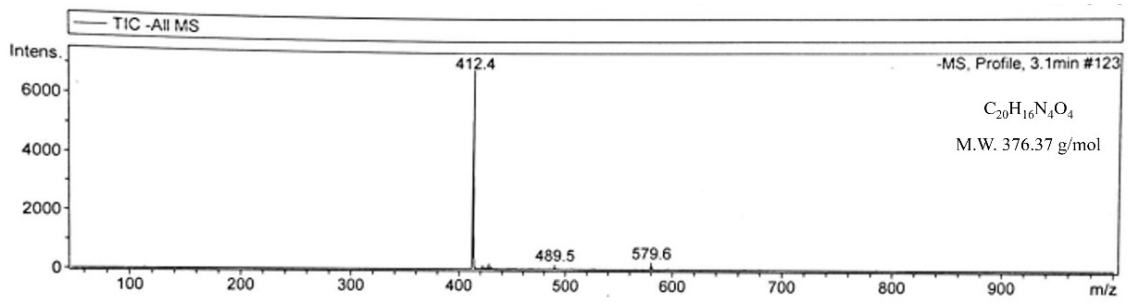


^{13}C NMR spectrum of compound 5h

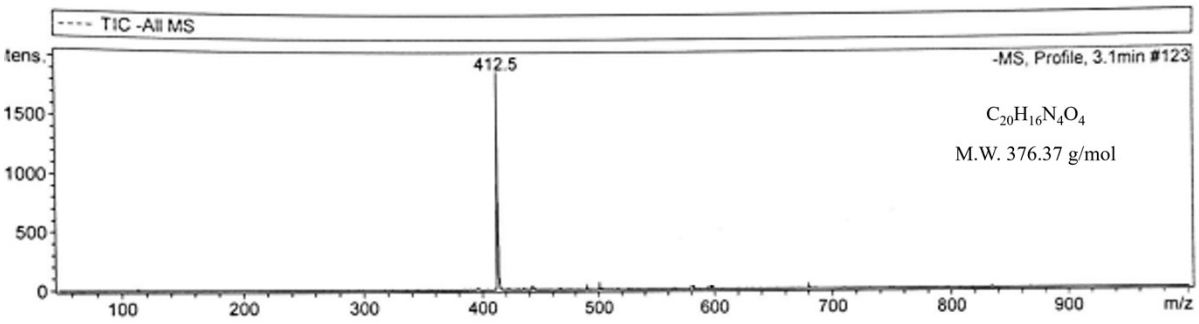


10 Mass spectrometry

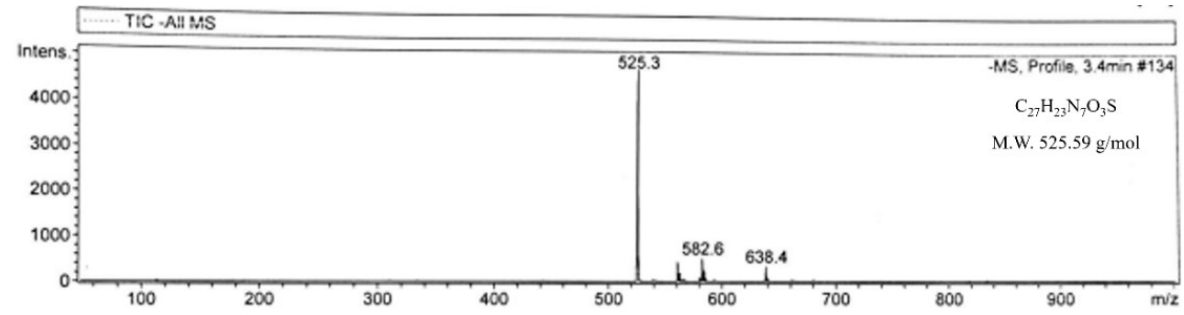
LCMS spectrum of 3a



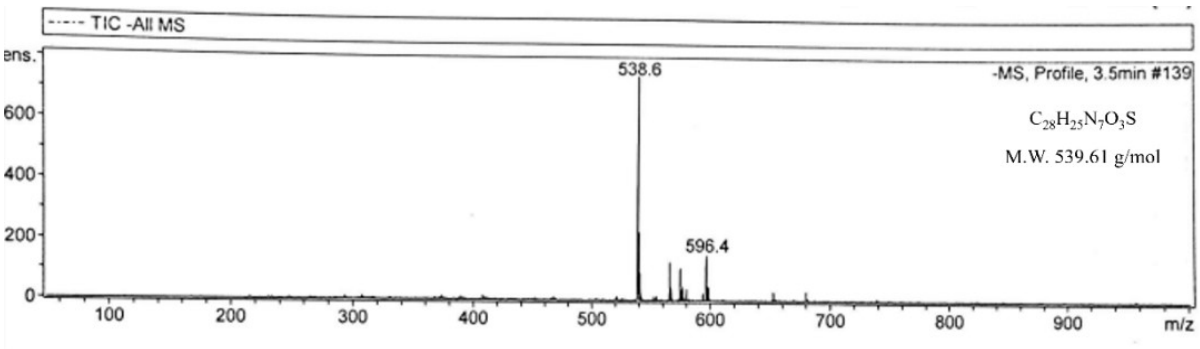
LCMS spectrum of 3b



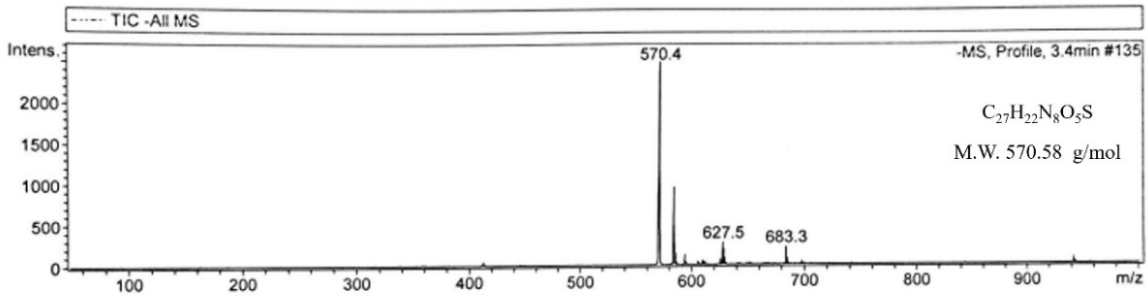
LCMS spectrum of 4a



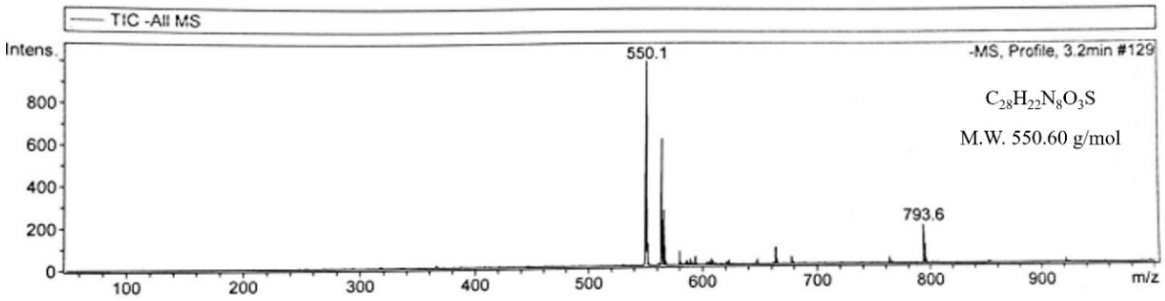
LCMS spectrum of 4b



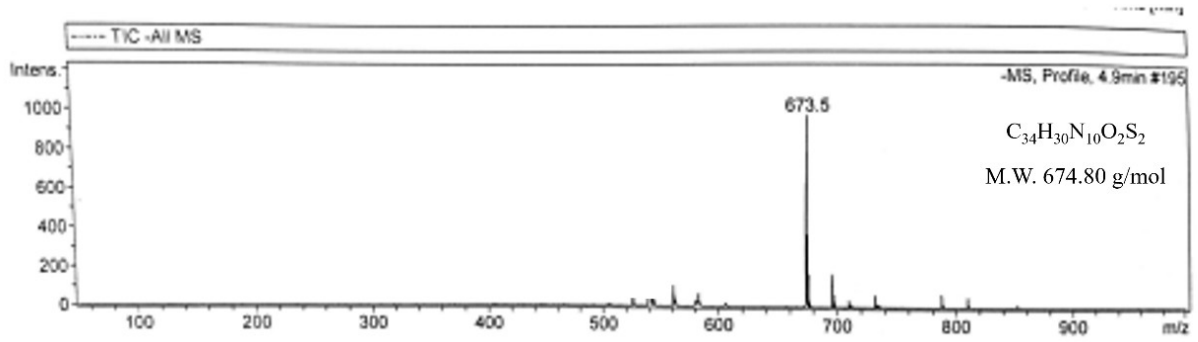
LCMS spectrum of 4c



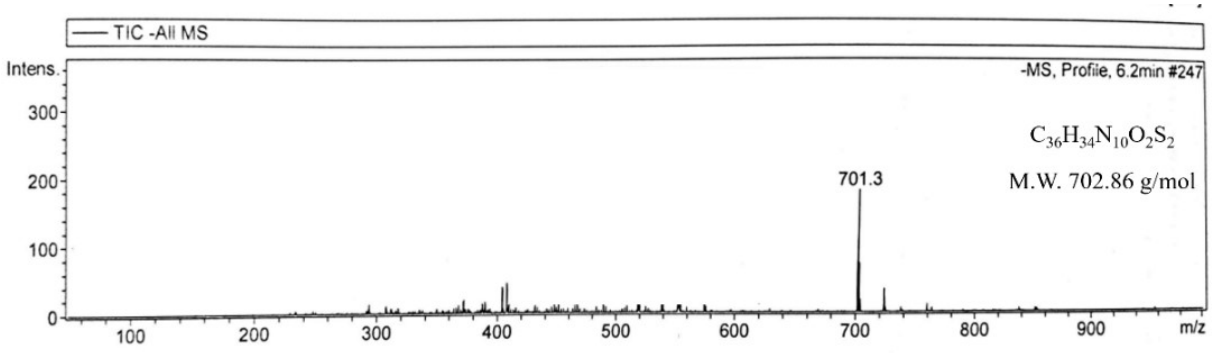
LCMS spectrum of 4d



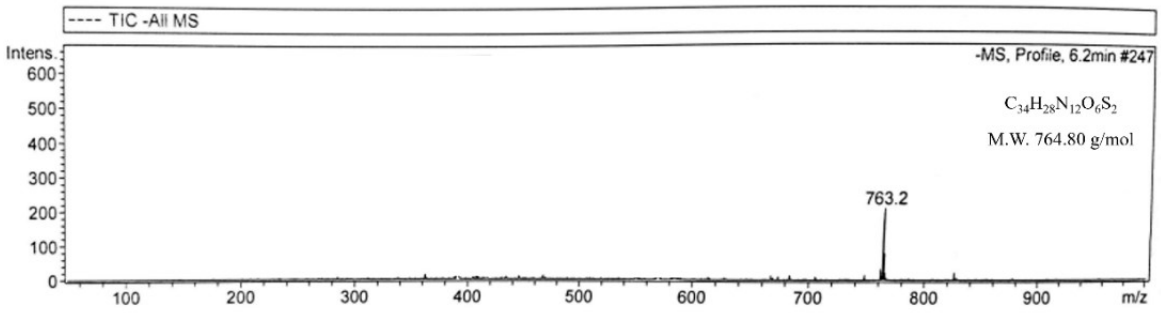
LCMS spectrum of 5a



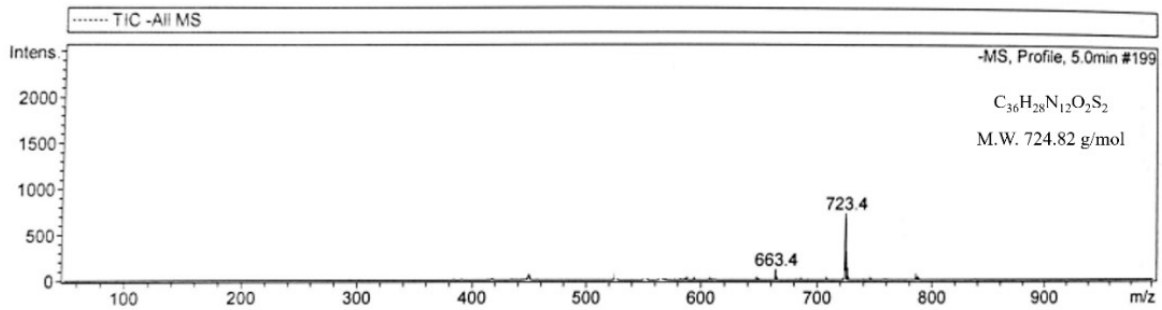
LCMS spectrum of 5b



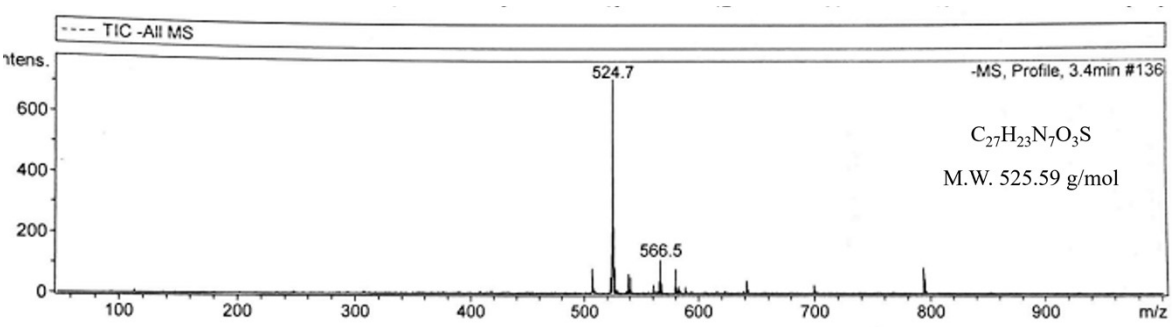
LCMS spectrum of 5c



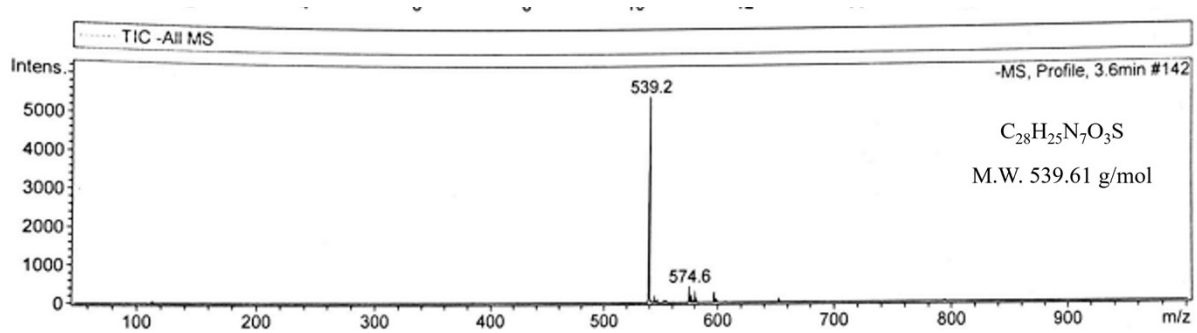
LCMS spectrum of 5d



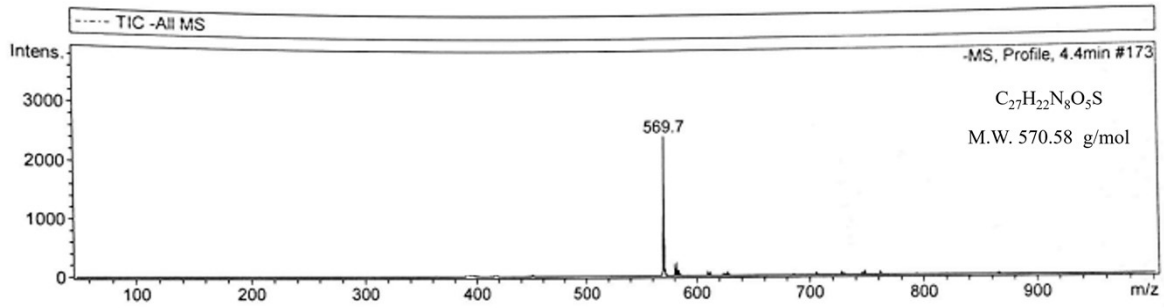
LCMS spectrum of 4e



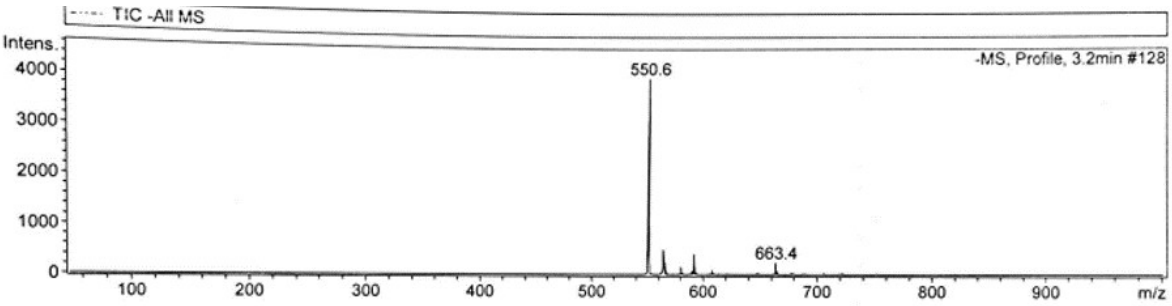
LCMS spectrum of 4f



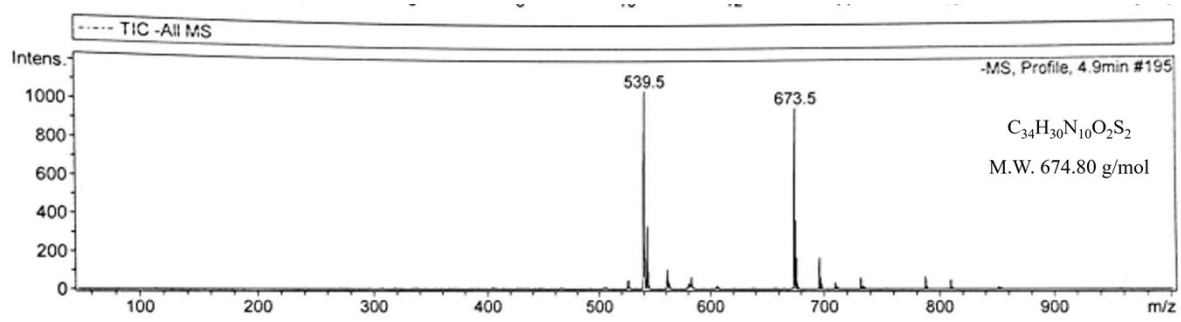
LCMS spectrum of 4g



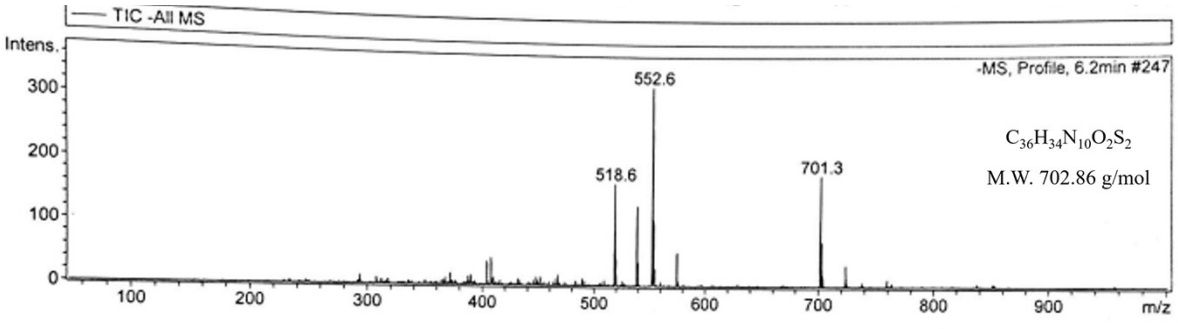
LCMS spectrum of 4h



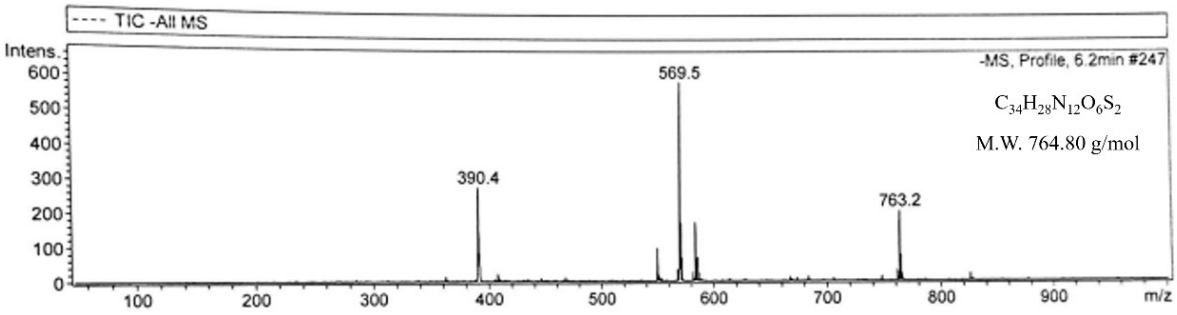
LCMS spectrum of 5e



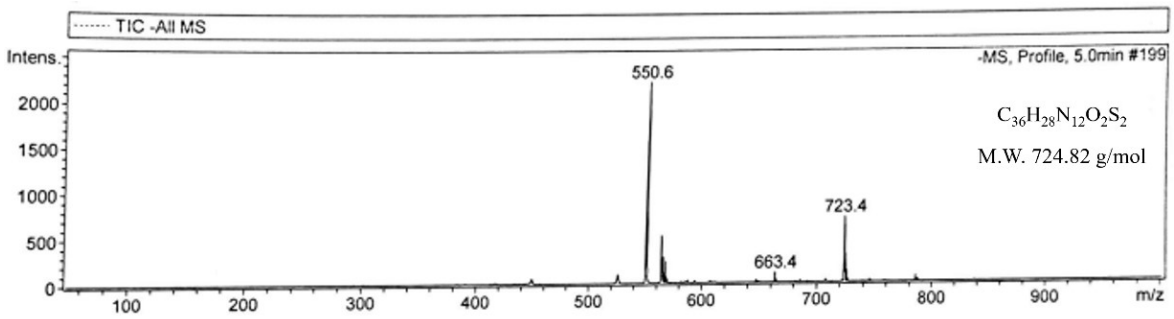
LCMS spectrum of 5f



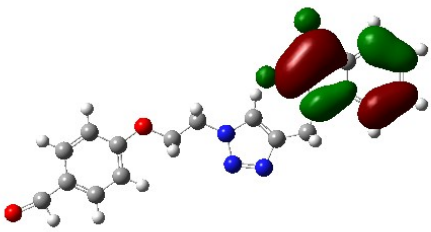
LCMS spectrum of 5g



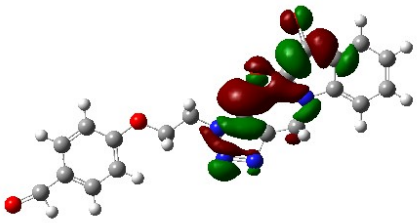
LCMS spectrum of 5h



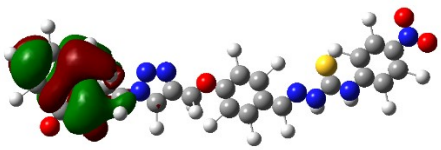
HOMO and LUMO of 3b, 4c, 4g and 5c



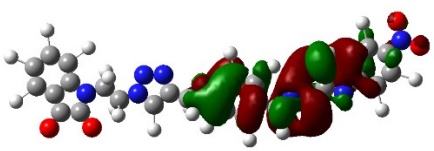
HOMO (3b)



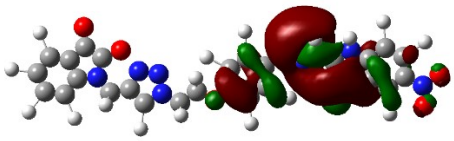
LUMO (3b)

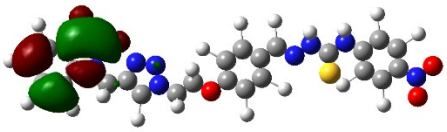


HOMO (4c)

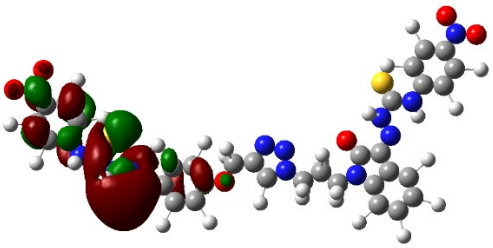


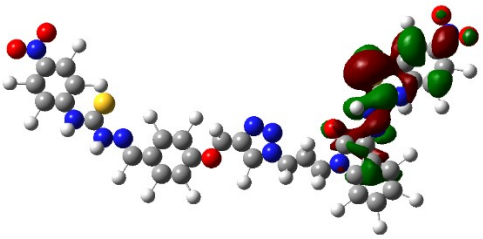
LUMO (4c)





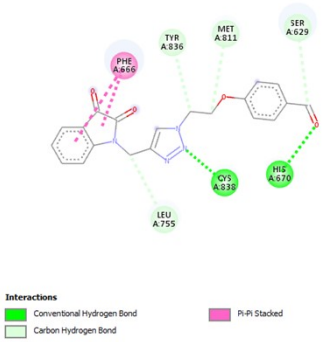
HOMO (4g) **LUMO (4g)**

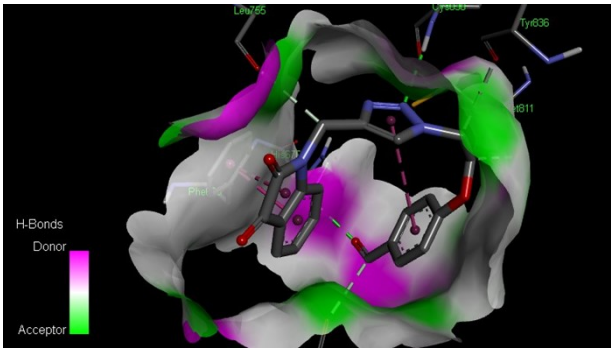




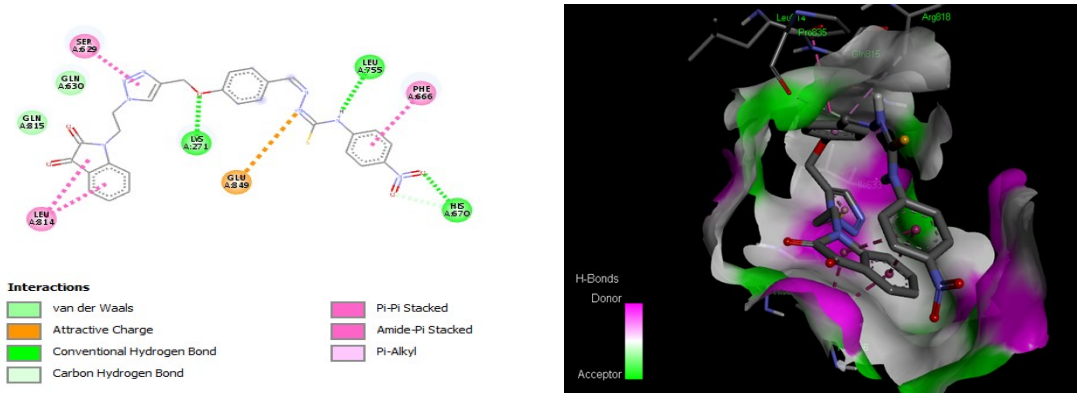
HOMO (5c) **LUMO (5c)**

2D and 3D representation of molecular interactions of the ligand molecules (3b, 4c, 4g and 5c) with various amino acid residues in the binding pocket of the protein PI3K

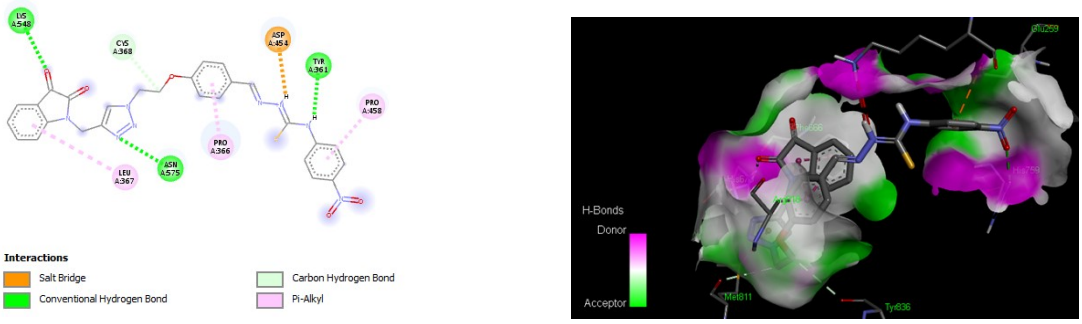




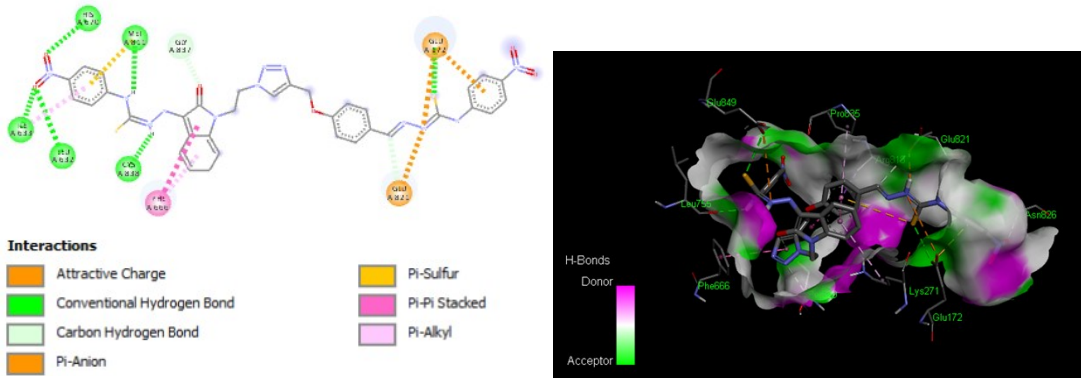
3b



4c



4g



5c

11 References

1. Shukla, A., Synthesis and biological screening of benzimidazole derivatives. *International Journal of Pharmaceutical sciences and Research* **2012,** *3* (3), 922.

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3. Kumar, S.; Bains, T.; Won Kim, A. S.; Tam, C.; Kim, J.; Cheng, L. W.; Land, K. M.; Debnath, A.; Kumar, V., Highly Potent 1H-1,2,3-Triazole-Tethered Isatin-Metronidazole Conjugates Against Anaerobic Foodborne, Waterborne, and Sexually-Transmitted Protozoal Parasites. *Frontiers in Cellular and Infection Microbiology* **2018,** *8*.
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5. Banday, A. H.; Shameem, S. A.; Gupta, B.; Kumar, H. S. J. S., D-ring substituted 1, 2, 3-triazolyl 20-keto pregnenanes as potential anticancer agents: Synthesis and biological evaluation. **2010,** *75* (12), 801-804.